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ELECTRICAL ACTIVITY IN THE UTERUS OF THE RAT[1]

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THE relationship between endocrine state and contractile activity of the uterus has been extensively studied; however, investigations pertaining to the influence of the sex hormones on the electrical activity underlying uterine movements are few. In the castrate the uterus is quiescent electrically and mechanically; however, estrogen raises the excitability of the uterus to the point that impulse propagation will occur (Bozler, 1937, 1938a, 1938b, 1941, 1948). Measurements of excitability of the uterus to shocks during the estrous cycle of the rat show that it is highest during late estrus and lowest in late diestrus and early proestrus (Katzenstein, 1938).

The present work was undertaken to contribute further information regarding the action of estrogen and progesterone on the electrical activity of the uterus.

MATERIALS AND METHODS

Eighty-two sexually mature virgin castrate rats of the Sprague-Dawley strain were used in this study. Seventy-two of the animals were pre-treated with an estrogenic substance: eighteen received diethylstilbestrol[3] and fifty-four received estradiol cyclopentylpropionate.[4] The injections were given subcutaneously in the abdominal region 24 hours apart.

Graded dosage experiments indicated that 10 μg./day of diethylstilbestrol for five days was the optimal dose. The optimal dose of estradiol was 5 μg./day for four days.

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[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Physiology in the Graduate College of the University of Illinois, 1953.

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[3] Metestrol, Metro.

[4] E. C. P., Upjohn.

With lower doses than these responses to shocks were inconsistent and with higher doses spontaneous electrical discharges tended to be continuous.

One group of seven and two groups of six rats were injected with one, five, and ten milligrams of progesterone[5] twelve hours after the last of four daily 5 μg. priming doses of estradiol. These animals were sacrificed at 1, 2, 4, 8, 12, and 24 hours after the administration of progesterone. Ten animals were used as castrate controls and either received no treatment or were injected with 0.5 cc. of corn oil.

For use, the uteri were removed under ether anesthesia and were suspended in a moist chamber kept at 37° C. Silver-silverchloride electrodes made contact with the uterus through a series of Ringer-agar wicks. Stimuli were provided by an electronic square wave generator. Electrical responses were amplified by a four-stage direct-coupled amplifier and recorded on one channel of a two-channel electrocardiograph ink-writer. The uterus was grounded between the recording and stimulating electrodes.

Contractions were recorded simultaneously by attaching one end of the uterus to the plate pin of an electronic transducer tube.[6] The output of the tube was amplified and fed onto the second pen of the ink-writer. An effort was made to maintain the tension on the uterus so that there would be no slack; that is, recording was essentially isometric. However, it was not possible to keep the same tension on the muscle at all times since the level of tone fluctuated considerably. In all cases where contractions were recorded, the upper line is the record of contraction and the lower line is the record of electrical activity.

In five experiments simultaneous records from three parts of the uterus were obtained with the aid of a multichannel crystograph ink-writer and high-gain A. C. amplification. In these experiments contractions were not recorded.

RESULTS

1. *The castrate uterus:* The excitability of the uterus began to decline soon after castration but spontaneous electrical and mechanical activity were present in some measure for about two weeks (Fig. 1 A–B). Between two and three weeks after castration spontaneous bursts of electrical activity and contractions were absent though large applied shocks elicited small contractions which were visibly localized about the stimulating electrodes (Fig. 1 C). Uteri of long-standing castration showed no spontaneous electrical or mechanical activity and did not respond to shocks (Fig. 1 D).

2. *The estrogen-primed uterus:* Graded doses of estrogen caused a return of the uterus to a high level of excitability. This excitability, as judged by the amount of spontaneous activity and responsiveness to shocks, was roughly proportional to the dose over the range administered. Generally, doses of estradiol more than 10 μg./day for four days produced continuous electrical discharge and sustained contraction (Fig. 2).

a. *Spontaneous activity:* All estrogen-treated uteri showed spontaneous activity from the start of the experiment, though with low doses of estradiol (0.01–0.1 μg./day) the magnitude of the electrical discharge was small, being less than a millivolt.

---

[5] Wolin's Crystalline Aqueous Suspension.

[6] R.C.A. 5734 Tube.

The spontaneous activity of the rat uterus was characterized by two types of electrical discharge, a low broad wave of less than a millivolt and of about 0.5 second duration, and a high fast spike of one to two millivolts and of about 0.1 second duration (Fig. 3). The small electrical discharge was usually continuous and not associated with a measurable contraction. The high spikes occurred in bursts of 15 to 30 spikes and were asso-

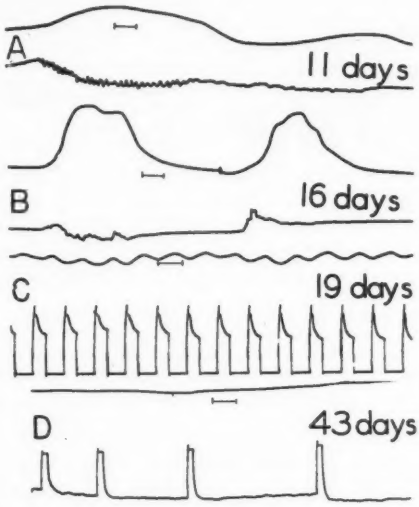

FIG. 1. Decline with time of spontaneous electrical and mechanical activity after ovariectomy. Lower tracing, electrical activity; upper tracing, mechanical activity. A and B, spontaneous activity. C, local mechanical activity in response to large shocks. Electrical record shows only stimulus artifacts. D, absence of spontaneous activity and of responsiveness to shocks; electrical record shows only stimulus artifacts. Time line = 1 second.

ciated with total contraction of the uterus. The high spikes preceded contraction and died out before relaxation was complete.

Multichannel recording from the excised uterus showed that different portions of the uterus could be simultaneously and independently active (Fig. 4). One part of the uterus could be quiescent while two other parts were active, one part only could be active, or all points from which records were being taken could be active with discharges occurring at different frequencies. Such local activity was always of the low type; however, when any one point showed massive rapid discharges, all other points from which records were being taken responded in a like manner after a lapse of time (Fig. 5). From these times, velocities of propagation were determined which varied from 0.26 to 6.0 cm./second.

In a preparation *in situ* (Fig. 6), all points from which records were being taken began their spontaneous discharges simultaneously. This could

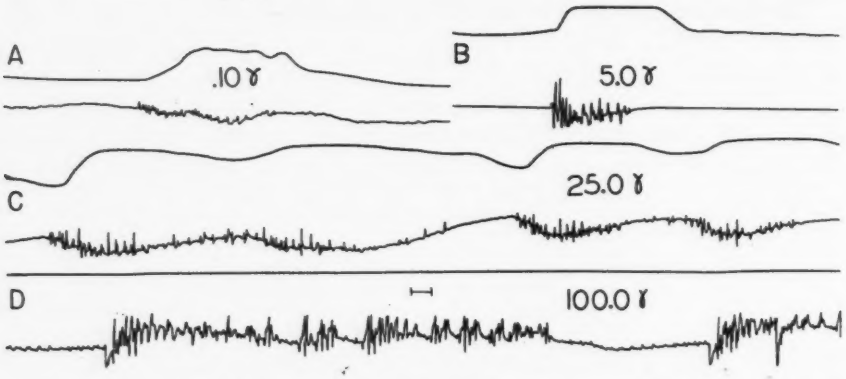

FIG. 2. Spontaneous electrical and mechanical activity at various dosages of estradiol. Upper tracing, contractions; lower tracing, electrical activity. A and B, rhythmic contractions and bursts of spike potentials. C, relaxation incomplete, electrical activity practically continuous. D, electrical activity continuous, contraction complete and sustained. Time line = 1 second.

have resulted from excitation of the myometrium by extrinsic nerves. Such an observation is in agreement with the statement of Reynolds and Kaminester (1935) that there is no propagated wave of contraction on pre-sacral nerve stimulation but that the uterus responds as a unit. In other respects the uterus *in situ* behaved like the excised uterus.

When a preparation exhibited rhythmic contractions and electrical discharges coincident with contraction, there was often a D.C. shift in the electrical baseline (Fig. 7). The shift began with contraction and returned to the level of origin when relaxation was complete. This suggested that the shift might be caused by electrode movement. However, measurements of interelectrode distances during contracted and relaxed states failed to reveal any difference. Also, mechanical tugging of the preparation did not elicit the shift.

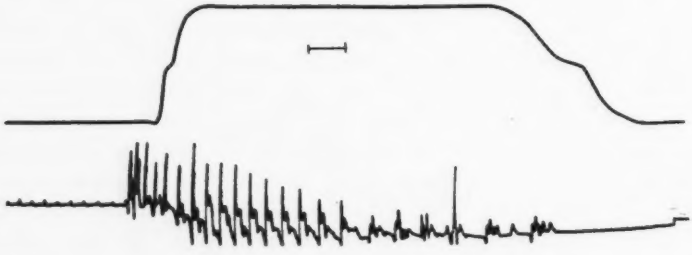

FIG. 3. Low level spontaneous activity not associated with measureable contraction followed by burst of spikes associated with total contraction. Lower tracing, electrical activity; upper tracing, mechanical activity. Time line = 1 second.

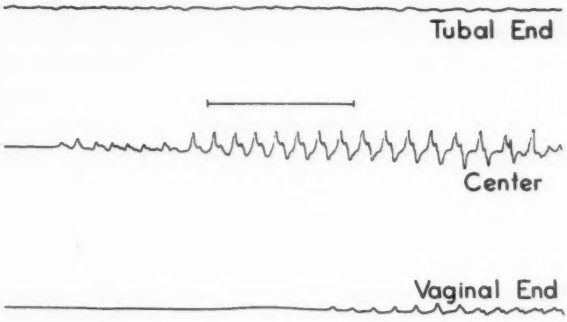

FIG. 4. Multichannel recording showing non-propagated spontaneous electrical discharges at center and vaginal end while tubal end is quiescent. Contractions not recorded. Time line = 1 second.

b. *Response to shocks:* In the uterus primed with a suboptimal amount of estrogen, the action potential travelled only a short distance before dying out (Fig. 8). In the uterus primed with an optimal amount of estrogen the action potential either travelled only a short distance or it was propagated throughout the uterus (Fig. 9).

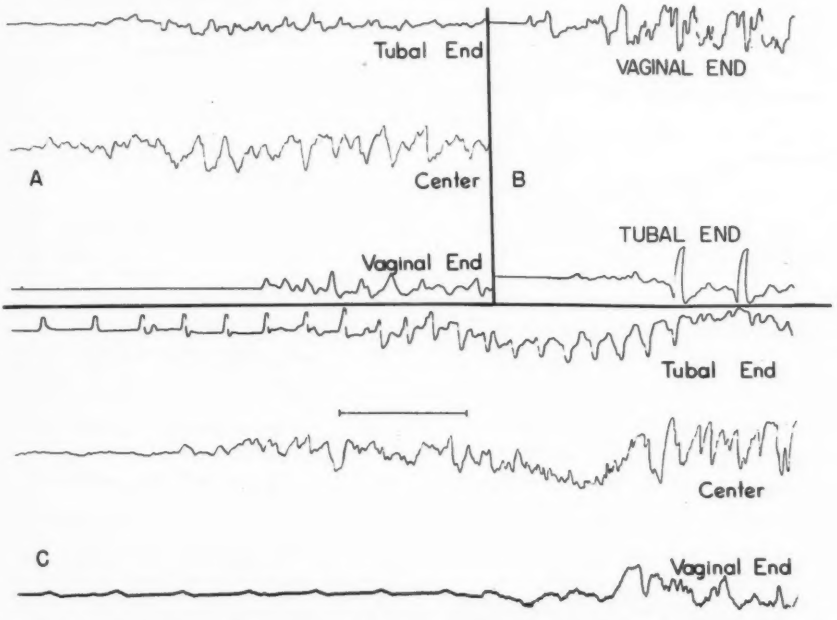

FIG. 5. Multichannel recording showing propagated spontaneous electrical activity beginning at different places on the uterus. A, electrical activity beginning at center of preparation. B, electrical activity beginning at vaginal end. C, electrical activity beginning at tubal end. Contractions not recorded. Time line = 1 second.

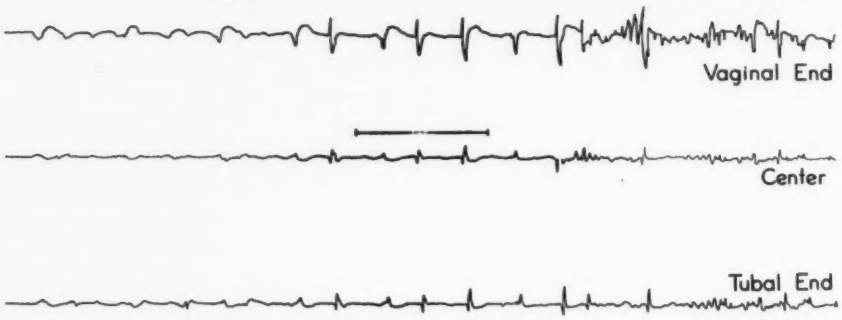

FIG. 6. Multichannel recording from uterus *in situ*. Spontaneous electrical discharges beginning simultaneously at vaginal end, center, and tubal end. Contractions not recorded. Time line = 1 second.

No two preparations responded with the same shape of action potential. Even within the same preparation the action potential varied from point to point and at the same point with time. The action potentials ranged in shape from a single diphasic wave to one having several components. (Fig. 9 F and G). Varying the distance between the recording electrodes failed to yield a characteristic action potential. Total contraction of the

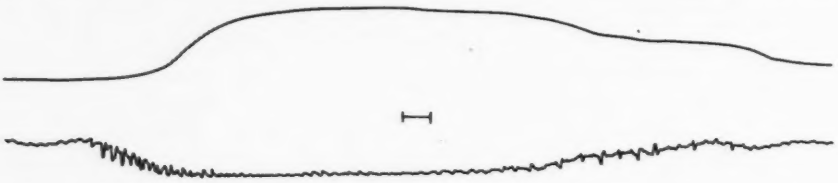

FIG. 7. Spontaneous burst of electrical activity (lower record) accompanied by contraction (upper record). Electrical record shows D.C. shift. Time line = 1 second.

uterus was always associated with repetitive electrical discharge; discrete single action potentials were not associated with measurable contractions.

The velocity of propagation of the action potentials elicited by shocks varied from 2 to 10 cm./second with a mean of 4.2 cm./second. Figure 9 A–C shows three responses at different conduction distances in one preparation to shocks of the same intensity and duration. The velocities of propagation of these three responses varied from 2.3 to 9.5 cm./second.

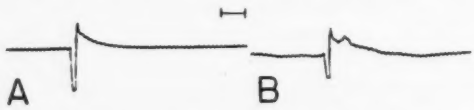

FIG. 8. Effect of an inadequate dose of estrogen for impulse conduction in the uterus. Preparation primed with a single 10 μg. dose of diethylstilbestrol. A, conduction distance = 8 mm., only stimulus artifact present. B, conduction distance = 6 mm., stimulus artifact followed by a small action potential. Time line = 1 second.

It was sometimes difficult to distinguish a response to a shock from a spontaneous discharge. For this reason, if spontaneous activity preceded the shock, the potentials following the shock were not used in the computation of velocity.

The latency of the response shortened on the application of repeated shocks at rates from 1 per second to 1 per three seconds (Fig. 9 D–F).

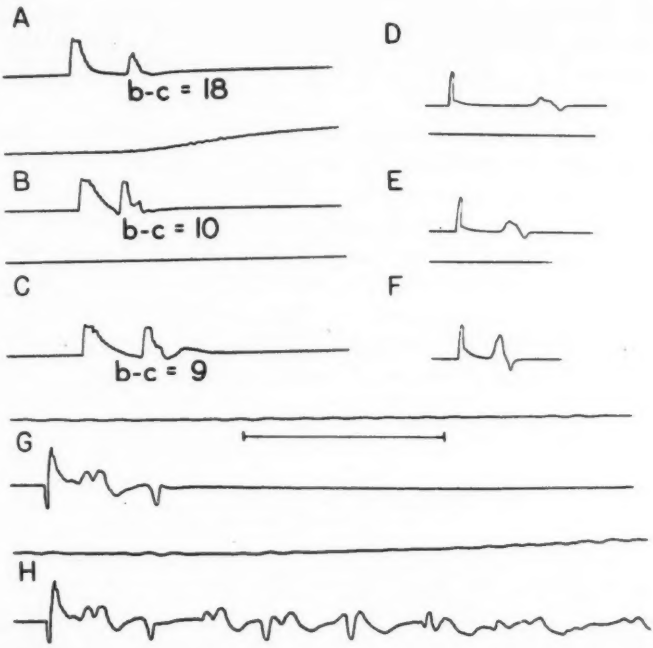

FIG. 9. Three separate preparations, A–C, D–F, and G–H, showing variability of wave form of action potential in response to single shocks. In all three preparations action potentials conducted throughout the entire uterus. A–C shows variability of latency to shocks of same intensity and duration at different conduction distances. b-c = conduction distance in millimeters. D–F shows facilitation of response to three consecutive identical shocks, conduction distance the same for all three responses. D, latency = 0.466 seconds; E, latency = 0.266 seconds; F, latency = 0.200 seconds. G–H shows action potential composed of several components. G, single response; H, repetitive response.

Measurements of muscle length showed that the reduced latency could not have been the result of shortening of conduction distance by contraction. The phenomenon appears to be a true facilitation of the response and can account, at least in part, for the variable conduction velocity.

Whether or not the uterus was contracted did not seem to affect its ability to propagate impulses. However, when the uterus was relaxed there was a tendency for one shock to set off a repetitive electrical discharge

whereas discrete potentials were more readily obtained when the muscle was contracted.

3. *Progesterone-treated uteri:* It was thought desirable to know whether or not progesterone would inhibit estrogen-induced excitability. Under the time-dosage conditions of these experiments, estrogen-primed, progesterone-treated uteri showed spontaneous electrical activity and responses to shocks which in no way differed from the controls (Fig. 10).

Four to eight hours after the administration of progesterone the uteri tended to relax beyond their original length following a contraction, necessitating a readjustment of the recording setup to restore the original level of tension.

4. *The effect of nicotine:* Soaking the uterus in nicotine in a concentration of 10^{-3} for 2 minutes reversibly blocked spontaneous electrical and mechanical activity. Soaking in nicotine 10^{-4} for 2 minutes had a marked stimulatory action (Fig. 11). Spontaneous electrical activity of the high type became continuous and contraction was complete and sustained. The spontaneous action potentials induced by nicotine 10^{-4} had a varying number of components which separated and then re-fused into a broad ragged wave (Fig. 11 C). If nicotine 10^{-4} was allowed to act for 30 minutes the high synchronous type of electrical discharge was abolished reversibly.

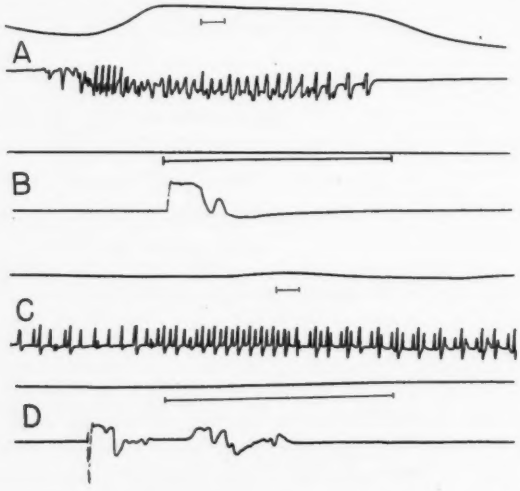

FIG. 10. Animal injected with 10 mg. progesterone 12 hours after the last of four daily 5 μg. priming doses of estradiol. A, spontaneous electrical activity (lower tracing) and contraction (upper tracing) 1 hour after the injection of progesterone. B, response to shock 1 hour after the injection of progesterone; shows large stimulus artifact followed by action potential. C, spontaneous electrical activity (lower tracing) associated with a period of maintained contraction (upper tracing) 24 hours after the injection of progesterone. D, response to shock 24 hours after the injection of progesterone. Shows stimulus artifact followed by complex action potential.

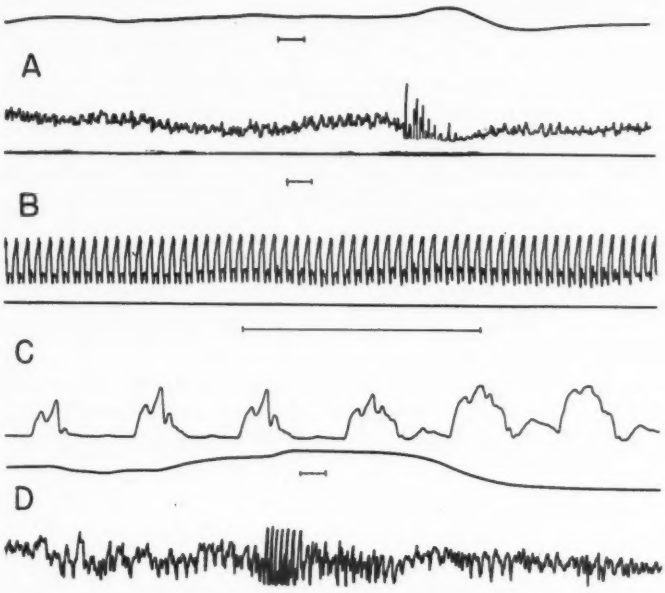

FIG. 11. Preparation primed with 5 μg./day of estradiol for four days. A, spontaneous activity before nicotine 10^{-4}; B, C, spontaneous activity after soaking in nicotine 10^{-4}, contraction sustained. D, spontaneous activity after washing for 35 minutes. Time marks = 1 second.

DISCUSSION

It is clear from the data collected in this study that estrogen transforms the uterus of the castrate rat from a practically inexcitable structure into a highly excitable one. This excitability is reflected in the spontaneous electrical discharges in the uterus and in its ability to propagate impulses in response to a shock.

The high propagated spikes are apparently caused by successive excitation of the myometrial cells, but what the conduction system is or how estrogen activates it is still not known. This system could be neural, but in order to account for the slow velocity of propagation, many synapses would have to be present. Such a ganglionic system has not been convincingly demonstrated in the uterine horn, though it is claimed by some to exist (Keiffer, 1932; Isidore, 1952). The high concentration of nicotine (10^{-3}) required for blockage of conduction argues against a ganglionic system. Bozler (1937) has presented evidence that in the uterus muscle is the tissue of conduction and that the uterine muscle is a syncytium. If the myometrium of the rat were a syncytium, it does not seem reasonable that the action potential in response to a shock would be of such a variable wave-form. In a continuous system a characteristic action potential would be expected.

In whatever manner the myometrial cells are organized and coordinated in their activity, repetitive electrical discharge precedes contraction and total contraction is accompanied by a burst of high sharp spikes. Measurements of the velocity of propagation of these action potentials agree well with those reported by Bozler (1938).

The slow electrical deflection (D.C. shift) is not easily explained. Balassa (1940) described such a slow deflection in the rabbit uterus *in situ* and attributed it to the fact that the uterus was dominated by progesterone. The D.C. shift in the rat uterus is not due to the dominance of progesterone, as it occurs in both progesterone and estrogen treated uteri. This phenomenon is perhaps caused by a fusion of spikes from individual myometrial cells discharging in rapid succession.

Upon repeated stimulation, impulse conduction in the rat uterus is facilitated. The facilitation occurs in all states of contraction and relaxation and is not due to shortening of conduction distance by contraction.

The experiments with progesterone indicate that this hormone has no effect on estrogen-induced excitability. It is seen, however, that progesterone affects the tone of the myometrium. It is concluded that progesterone may affect the contractile mechanism but does not affect the ability of the uterus to propagate impulses.

SUMMARY

The electrical and mechanical activity of the rat uterus was studied. Spontaneous electrical and mechanical activity and responsiveness to shocks were absent in the uteri of castrated rats. Estrogens restored the level of excitability in the uteri of such animals to a high level.

Spontaneous electrical discharges in the estrogen-primed uterus were of two types, a low broad wave of less than one millivolt and a high fast spike of one to two millivolts. The low magnitude potential was independent in different portions of the uterus and the high spikes were propagated throughout the uterus. During spontaneous activity there was often a slow D.C. deflection in the electrical baseline. Spontaneous activity may be initiated at any point on the uterus and spread in all directions.

The wave form of the propagated action potential in response to a shock is variable from point to point and from time to time within the same uterus. The shape of the action potential also varies from one preparation to another.

The velocity of propagation of the action potential in response to a shock has a mean value of 4.2 cm./second. Upon repeated shocks, the velocity of conduction increases.

Progesterone had no effect on estrogen-induced excitability. Nicotine in a concentration of 10^{-3} reversibly blocked spontaneous electrical activity while a concentration of 10^{-4} had a marked stimulatory action.

Acknowledgment

The author would like to express his gratitude to Professor C. Ladd Prosser for suggesting the problem and for his advice and counsel throughout the course of the work.

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AN *IN VITRO* ASSAY FOR ESTRADIOL-17β AND ESTRONE[1]

EDWIN E. GORDON AND CLAUDE A. VILLEE

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ESTRADIOL-17β[2] added *in vitro* has been shown to stimulate a reaction mediated by a diphosphopyridine nucleotide (DPN) dependent isocitric dehydrogenase in human, term placental extracts (Villee, 1955; Villee and Gordon, 1955). In the course of this reaction, DPN is reduced to DPNH and the product can be measured by its absorption of light at 340 mμ. in a spectrophotometer (Gordon and Villee, 1955). In this paper, the rate of DPN reduction is shown to be a function of the concentration of estradiol in the incubation medium. Estrone is as effective as estradiol in this reaction, but none of a number of other steroids tested has this marked stimulatory property. These circumstances provide the basis for a sensitive, *in vitro* method for measuring estradiol and estrone. Data are presented which compare the analyses of urine extracts by this method of estrogen determination with those of bioassay by the vaginal smear response in adult castrate rats (Smith and Smith, 1952). Kinetic analysis of the data is compatible with the hypothesis that estradiol functions to activate the DPN dependent isocitric dehydrogenase.

MATERIALS

Placental extract ($S_{57,000}$). Human, term placentas were obtained as soon as possible after delivery, cut into 3–5 cm. pieces and washed in ice cold 0.9% saline solution. These pieces were sliced, dried on absorbent paper and the gross connective tissue was dissected away. The tissue was homogenized in cold 0.25 M sucrose (20% weight per volume) in a smooth glass homogenizer fitted with a Teflon pestle. Separation of the cellular debris and residual connective tissue was effected by a preliminary 2000×g centrifugation at 5° for 10 minutes. The supernatant fluid was centrifuged at 57,000×g for 60 minutes in a Spinco model L preparative ultracentrifuge to remove the mitochondria and microsomes. The clear, reddish fluid ($S_{57,000}$) was stored at 0° until used. This $S_{57,000}$ fraction retains maximum activity for at least 6 to 8 hours.

Steroid suspensions. At room temperature, some proteins of the $S_{57,000}$ fraction were precipitated when low concentrations of ethanol were present. Therefore, all additions to the incubation mixture were made up in an aqueous medium. Stock solutions of steroids were prepared by dissolving 3.0 mg. of the steroid in 10 ml. of 95% ethanol.

Received June 14, 1955.

[1] This investigation was supported by grants from the Charles A. King and Marjorie King Fund, from Winthrop-Stearns, Inc., and by research grant C2400 from the National Institutes of Health, U. S. Public Health Service.

[2] The estradiol used in all experiments was the 17β isomer unless otherwise indicated.

One ml. of this solution was then transferred to a smooth glass homogenizing vessel and the alcohol was removed by directing a stream of filtered, compressed air over the solution. When perfectly dry, the crystalline steroid can be seen to adhere to the wall of the vessel. Ten ml. of distilled water were added to the vessel and a fine suspension was prepared by homogenizing for 2 to 3 minutes with a Teflon pestle. When only small quantities of steroid were available, 0.3 mg. was weighed directly into a homogenizing vessel and a suspension containing 30 μg. per ml. was prepared as described above. Appropriate concentrations of steroid suspensions were made by diluting this stock suspension. The alcoholic solutions and the aqueous suspensions were stored at 5° C. with precautions to avoid evaporation of the solvent; no loss in acitvity was noted after 5 months' storage.

Suspensions of unknowns. Aqueous suspensions of the alcoholic urine extracts were prepared for assay by removing the alcohol and homogenizing the residue with distilled water.

Buffer. 0.3 M tris(hydroxymethyl)aminomethane (Tris) buffer was acidified to pH 7.4 and stored at 5° C.

Substrate. dl-Na_3 isocitrate obtained from the H. M. Chemical Company, Santa Monica, California, was dissolved in distilled water and neutralized with 0.1 N HCl using phenol red as indicator. The solution was prepared daily. The final concentration of substrate in the reaction vessel was 2 micromoles per ml.

7.5 mM DPN. 25 mg. of DPN (Pabst, 95% pure) was dissolved in 5 ml. of distilled water. The solution was stored at 5° C.

0.1 M $CoCl_2$. When stored at 5° C. this solution keeps indefinitely.

METHOD

The reduction of DPN was measured in a Beckman model DU spectrophotometer at a wavelength of 340 mμ. The reaction mixtures were incubated at room temperature in quartz cuvettes with a 1 cm. light path. Three cuvettes—blank, control, and estrogen—were used to make one measurement of estrogen effect on a given $S_{57,000}$ preparation. Each cuvette received 1.0 ml. of 0.3 M Tris buffer, 0.1 ml. of 0.1 M $CoCl_2$ and 0.3 ml. of 0.02 M isocitrate. Since small changes in the concentration of Co^{++} produce marked variations in the reaction rate, suitable quantities of the buffer, substrate and cobalt solutions were mixed together and 1.4 ml. of the mixture was placed in each cuvette. The solution or suspension to be tested for estrogenic activity (0.1 to 0.5 ml.) was added to the estrogen cuvette. One ml. of $S_{57,000}$ preparation and enough water to give a final volume of 3.0 ml. was pipetted into each of the three cuvettes. One-tenth ml. of the DPN solution was added to the control and estrogen cuvettes to start the reaction and the cuvettes were inverted several times to mix the constituents. The optical density at 340 mμ. of each cuvette was recorded exactly one minute after mixing and again exactly 20 minutes after mixing.

For each placental preparation, a standard curve was constructed with five different concentrations of estradiol in the range 0.5 μg. to 0.075 μg. per cuvette. At each concentration of estradiol the amount of DPN reduced was expressed as the optical density reading 20 minutes after mixing, minus the optical density reading one minute after mixing. The amount of DPN reduced in the absence of added estradiol was computed in the same manner. Net DPN reduction for a particular concentration of estradiol was calculated by subtracting this assay blank. This net DPN reduction was plotted as a function of the amount of estradiol added (Fig. 1). The reaction mixtures containing the solution or suspension of unknown activity were treated in the same manner and the estradiol equivalent of the test material was calculated by interpolation from the standard curve. When the activity of the unknown sample exceeded 0.5 μg. of estradiol per

cuvette, the original sample was diluted and the activity was again determined at the lower concentration. This precaution is essential because of the non-linear relationship at high concentrations of estradiol.

RESULTS

The rate of DPN reduction in this system is dependent upon the concentration of estradiol in the incubation medium (Fig. 2). Three phases of

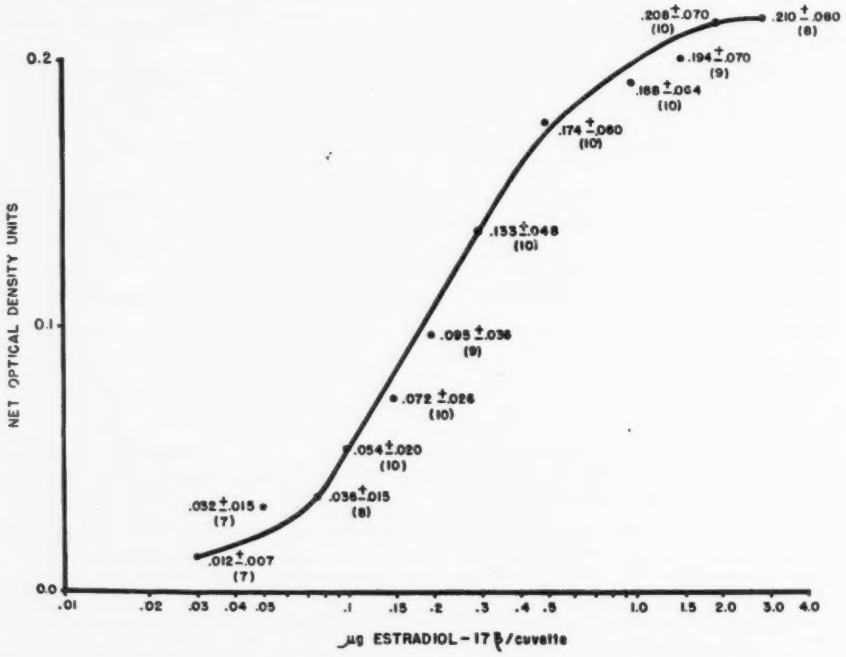

FIG. 1. DPN reduction by the placental $S_{57,000}$ fraction as a function of estradiol concentration. Net optical density units (ordinate) is defined in the text. The numbers are the means ± standard error of determinations with a number ( ) of different placental preparations.

DPN reduction can be distinguished: 1) an initial, slow, equilibration phase which proceeds for about 5 minutes, 2) a rapid, linear phase, and 3) a declining phase after 25 minutes of incubation. Because the amount of DPN reduced after 20 minutes in all our experiments fell within the second or linear range, the optical density at this time was chosen to represent estradiol stimulation of DPNH production. The DPN reduced in 20 minutes was plotted over a wide range of estradiol concentrations on semi-logarithmic paper (Fig. 1). At either extreme of the concentration scale, the reduction of DPN was not proportional to estradiol concentration and an S shaped curve resulted. An analysis of the data by the method

of Bliss (1944) revealed an index of precision (λ) of 0.106 when the response to four different estradiol concentrations (0.5, 0.3, 0.15, 0.10 μg. per cuvette) was compared in alternate series of experiments using different placental preparations in each series.

Several other steroids (Table 1) were tested with this system in order to determine the specificity of the stimulatory action of estradiol. Estrone

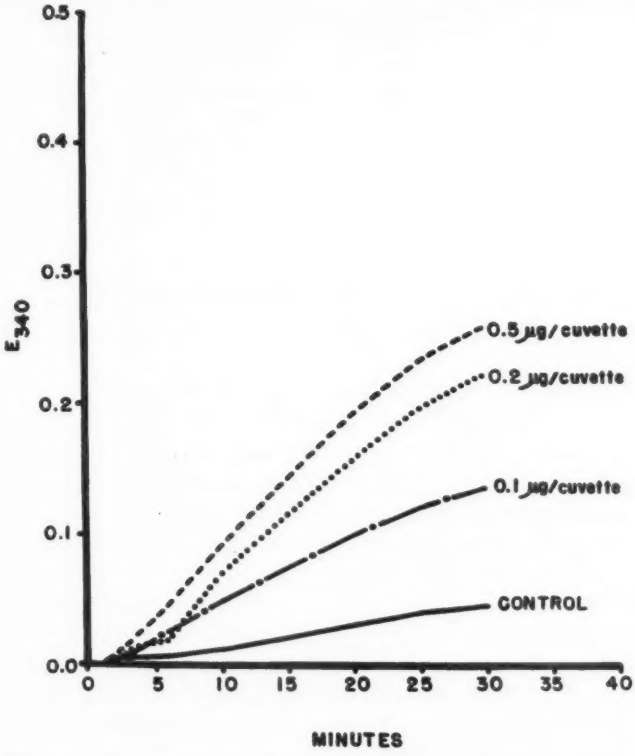

FIG. 2. The rate of DPN reduction by the placental $S_{57,000}$ in the absence and presence of 3 concentrations of estradiol. The initial one minute reading at each level of estradiol is subtracted from subsequent readings.

was found to have approximately 90% of the activity of estradiol. Of the other compounds tested, only testosterone, cortisol and 17α-ethinyl estradiol had measurable stimulatory effects on the rate of DPN reduction when added to the incubation medium in extremely large quantities. In a series of experiments designed to test the possibility of competition between hormones, 0.3 μg. estradiol per cuvette was added to reaction mixtures in combination with estriol, with progesterone, with cortisol and with testosterone at levels of 12 μg. per cuvette (Table 1). Estriol in combination with estradiol decreased DPN reduction by about 15%.

TABLE 1. THE EFFECT OF STEROIDS, SINGLY AND IN COMBINATION, ON DPN REDUCTION BY THE PLACENTAL $S_{57,000}$ FRACTION

Steroid	μg./cuvette	ΔO.D. at 340 mμ*	Steroid pairs	μg./cuvette	ΔO.D. at 340 mμ*
Control	—	.012	Control	—	.014
Estradiol	0.3	.224	Estradiol	0.3	.190
Estrone	0.3	.190	Estradiol and Estriol	0.3 12.0	.160
Estriol	15.0	.006			
17α-Ethinyl estradiol	15.0	.084	Estradiol and Progesterone	0.3 12.0	.201
Stilbestrol	15.0	.016			
Estradiol-17α	15.0	−.004	Estradiol and Cortisol	0.3 12.0	.212
Progesterone	15.0	.016			
Testosterone	15.0	.167	Estradiol and Testosterone	0.3 15.0	.273
Cortisone	15.0	.006			
Cortisol	15.0	.046			
Cholesterol	15.0	.015			

* The results are in optical density units obtained by subtracting the one minute reading from the 20 minute reading. Each value represents the mean of experiments with two or more different placental $S_{57,000}$ preparations.

Combination of estradiol with testosterone and cortisol resulted in an additive effect on DPN reduction. Progesterone did not affect DPNH production by estradiol.

The amount of estradiol plus estrone in alcoholic extracts of human urine fractionated by the method of Smith and Smith (1952) was determined. In Table 2, values derived from the rat vaginal smear assay (Smith and Smith, 1952) and *in vitro* placental $S_{57,000}$ assay are compared. In those fractions containing estradiol and estrone the results arrived at by the two methods are in good agreement. In contrast, the estriol urine fractions had no activity by our method.

The addition of 0.5 ml. of blood plasma, serum, or dialyzed serum to the reaction mixture inhibited DPN reduction. When 30 μg. of estradiol were added to a reaction mixture containing either of the blood fractions, little DPN reduction was observed. It is likely that the plasma and serum act either to bind the hormone so as to make it unavailable or to inactivate the placental isocitric dehydrogenase.

TABLE 2. COMPARISON OF ESTROGEN CONTENT OF URINE FRACTIONS BY A RAT VAGINAL SMEAR ASSAY (SMITH & SMITH, 1952) AND THE *in vitro* PLACENTAL EXTRACT TECHNIQUE

Urine fraction	Hormone present	μg. of hormone/ml.	
		Placental extract technique*	Rat vaginal smear assay
1	Estradiol	0.60	0.50 to 0.59
2	Estrone	3.87†	4.0 to 5.2
3	Estriol	0.00	200 to 250
4	Estriol	0.00	200 to 250
5	Estradiol	4.70	3.35 to 4.50
6	Estradiol	1.12	0.67 to 0.78

* Mean of determinations with two different placental preparations.
† Expressed as μg. estradiol/ml.

Kinetic Analysis

Estradiol has been shown to stimulate a DPN dependent isocitric dehydrogenase in the placental $S_{57,000}$ fraction (Villee, 1955; Villee & Gordon, 1955). In the present experiments the substrate has been present in excess, and all other constituents of the reaction mixtures have been kept constant. The velocity of the reaction mediated by this enzyme is a function of estradiol concentration in the incubation mixture (Fig. 1). The data suggest that the estrogen serves to activate the isocitric dehydrogenase. We can

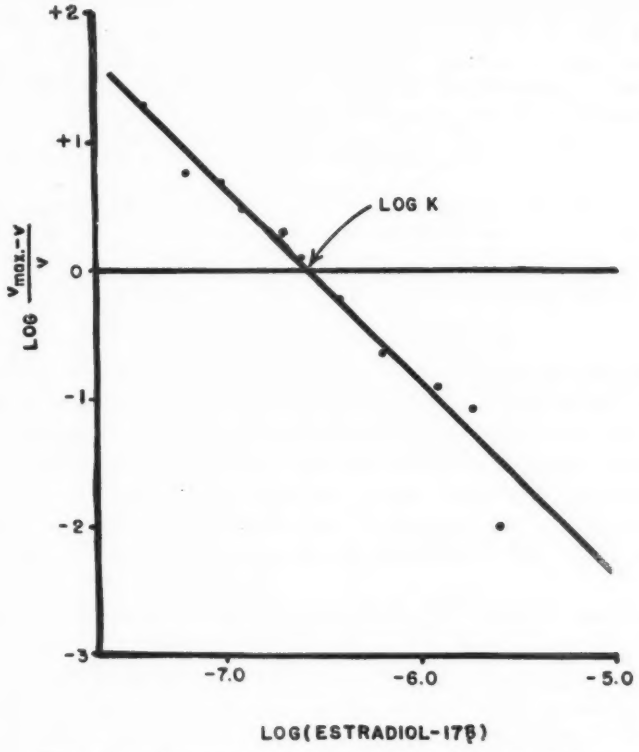

FIG. 3. Log $(v_{max}-v)/v$ vs. log. D. See text for discussion.

hypothesize that the enzyme exists in two states: 1) an inactive form (E′) and 2) an active form (E). We can then assume that estradiol (D) serves to convert the inactive to the active form. From Figure 2 it is seen that this activation reaction is not rate limiting after the initial equilibrium phase, and the reactions

$$E' + D \rightleftharpoons E \qquad (1)$$

may be considered to exist in equilibrium. The equation for the equilibrium constant of this activation may be written as

$$K = \frac{[E'][D]}{[E]} \qquad (2)$$

Under the stated experimental conditions, that is, with the substrate in excess, the

velocity of DPN reduction is proportional to the concentration of active enzyme (E):

$$v = K[E] \tag{3}$$

In terms of the above hypothesis, the velocity reflects the fraction of the enzyme in the active form. When the concentration of estradiol in the incubation medium is very large, virtually all of the enzyme would be in the active form and a maximum velocity (v_{max}) would be observed. Then

$$v_{max} = K[E_t] \tag{4}$$

where (E_t) refers to total enzyme concentration:

$$[E_t] = [E] + [E'] \tag{5}$$

Substituting equations 1, 3, 4, and 5 into equation (2) and converting to the logarithmic form gives:

$$\log K' = \log D + \log \frac{v_{max} - v}{v} \tag{6}$$

From equation (6), it is seen that a plot of $\log (v_{max} - v)/v$ vs. $\log D$ results in a straight line with a slope of -1 and an intercept on the log D axis of log K. When the experimental data are so plotted (Fig. 3), the slope of the straight line is found to be -1 and K is determined to be 2.82×10^{-7} moles per liter.

DISCUSSION

This method of estrogen assay provides a rapid, sensitive, *in vitro* technique for measuring the sum of the amounts of estradiol and estrone in samples without appreciable interference by other steroid hormones. All experiments reported in this paper were carried out with freshly prepared $S_{57,000}$ fractions. However, $S_{57,000}$ fractions which have been lyophilyzed and then redissolved respond to estradiol in the same manner. When stored at $-20°$, the lyophilyzed powder retains its activity for at least 4 months.

Stimulation of the DPN dependent isocitric dehydrogenase as determined by chemical methods have shown testosterone, progesterone, estriol and stilbestrol to be only 1/1000 as active as estradiol (Villee, 1955). The fact that testosterone is about 1/75th as active as estradiol when DPNH production is measured, suggests that this hormone stimulates some other DPN dependent enzyme, or that the hormone itself is enzymatically oxidized in the presence of DPN by the placental extract. Credence is given to this latter possibility by the work of Talalay, *et al.* (1955). These workers isolated a β-hydroxysteroid dehydrogenase from *Pseudomonas* which catalyzed the oxidation of testosterone with the formation of DPNH as one of the end products.

SUMMARY

A rapid, sensitive, *in vitro* technique for the determination of estradiol-17β plus estrone has been presented. This estrogen assay is based on the fact that the reaction mediated by a DPN dependent isocitric dehydrogenase of human placental extracts is accelerated by these hormones.

Several other steroid hormones were tested and found to have no appreciable effect on the reaction. The estradiol and estrone content of urine fractions as determined by this method was compared with results obtained by the rat vaginal smear technique; good agreement between the results of the two assays was obtained.

A hypothesis for the mechanism of action of estradiol in this system was proposed and the data were subjected to kinetic analysis.

Acknowledgments

The authors are indebted to Dr. O. W. Smith for the fractionated urine extracts and for assaying these extracts by the rat vaginal smear technique. We are also indebted to Dr. F. Snell of the Department of Biological Chemistry, Harvard Medical School for his assistance in the kinetic interpretation of the data. The cortisone and cortisol as free alcohols were kindly supplied by Dr. F. K. Heath of Merck and Co., Inc. We wish to thank Dr. F. L. Hisaw for a supply of estriol and Dr. L. L. Engel for the 17α-ethinyl estradiol and estradiol-17∝.

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THE INFLUENCE OF THE ADRENAL MEDULLA ON MUSCLE GLYCOGEN IN NORMAL AND HYPOPHYSECTOMIZED RATS

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IN RECENT years it has become more and more apparent that numerous hormones are influential in determining the level of glycogen in skeletal muscle. Epinephrine (Cori and Cori, 1928), adrenal cortical hormones (Long, Katzin, and Fry, 1940), growth hormone (Russell and Wilhelmi, 1950), testosterone (Leonard, 1952), and estradiol (Leonard, 1953) are among those that have been implicated. Antagonistic (Winternitz and Long, 1952) and additive effects (Bowman, 1953) have been demonstrated between several of these hormones, and it is becoming apparent that considerable interplay prevails.

It has not always been recognized in experiments dealing with the glycogen controlling hormones that antagonisms do exist. Especially has this been true in work concerned with the effects of adrenalectomy on glycogen stores. In such experiments (Long *et al.*, 1940) the fact that epinephrine is lost, as well as the cortical substances, generally has been overlooked. One probable reason for this is the finding that normal and adrenaldemedullated rats behave similarly in a number of physiological respects (Ingle and Corwin, 1939; Harris and Ingle, 1940), and it is sometimes assumed (Turner, 1955) that epinephrine is not being released in sufficient amounts, under resting conditions, to affect the functioning of the organism. However, it is doubtful if the period of fasting that usually precedes the analysis for muscle glycogen can be considered as a resting condition. That under this circumstance the adrenal medulla is effectively active, as regards muscle glycogen, will be shown in the present experiments.

MATERIALS AND METHODS

Male and female rats of the Long-Evans strain with initial weights of 150–200 grams were used. A standard diet of calf meal and water was fed *ad libitum*. All hypophysectomized animals were offered a 10% glucose solution, and adrenalectomized rats 1% NaCl in addition to regular drinking water. Adrenalectomized-hypophysectomized animals received both the salt and glucose solutions, and adrenaldemedullated rats were offered salt water for a period of two to three weeks after enucleation.

The operations were carried out under ether anesthesia using standard procedures. In experiments involving removal of the pituitary as well as the adrenal the two operations were carried out three days apart. With the males, the pituitaries were removed

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first, and with the females adrenalectomy was the initial operation. Adrenaldemedullated animals were not used nor subjected to hypophysectomy until 30 days after demedullation.

The rats received no food or glucose during the 24 hours previous to autopsy. Then, with the animals under Nembutal anesthesia (6 mg. per 100 gm. body weight), three muscles were taken for glycogen determination from each animal. The muscles were removed in the following order: rectus femoris, a section of the abdominal muscle wall, and one half of the diaphragm. The muscles were weighed immediately, placed in tubes containing 30% KOH, and allowed to digest completely in a boiling water bath. Details of these procedures and of the method of subsequent glycogen determination by the anthrone method are available in a previous report (Bowman, 1953). The glycogen values are presented as milligrams of glucose per 100 gm. of muscle, wet weight.

EXPERIMENTAL

In order to determine whether or not epinephrine is secreted under "resting conditions" in amounts sufficient to affect muscle glycogen, gly-

TABLE 1. EFFECT OF THE PITUITARY, ADRENAL CORTEX, AND ADRENAL MEDULLA ON MUSCLE GLYCOGEN IN FEMALE RATS

Treatment*	No. of animals	Rectus femoris	Abdominal muscle	Diaphragm muscle
		(Glycogen (mg./100 gm. muscle)		
Normal	18	429 ± 15†	506 ± 21	265 ± 19
Adrenaldemedullated	5	493 ± 13	596 ± 16	407 ± 12
Adrenalectomized	6	417 ± 4	453 ± 26	365 ± 9
Hypophysectomized	17	236 ± 12	288 ± 12	164 ± 11
Hypophysectomized-adrenaldemedullated	11	327 ± 9	427 ± 21	306 ± 16
Hypophysectomized-adrenalectomized	6	354 ± 4	358 ± 15	267 ± 13

* All values are from 24 hour fasted animals.
† Standard error of the mean.

cogen values in normal female rats were compared to those in adrenalectomized and adrenaldemedullated animals. It has been suggested (Leonard and Ringler, 1954) that growth hormone antagonizes the action of injected epinephrine, and thus the effect on muscle glycogen of adrenalectomy and adrenalmedullectomy was ascertained in hypophysectomized animals also. This should give some indication of the *in vivo* modification exerted by the pituitary on endogenous epinephrine activity. Four to six days after the last operation the animals were deprived of food, and the next day the glycogen determinations were made. All data from the above experiments are included in Table 1.

Intact rats. 1) Removal of the adrenal medulla resulted in glycogen levels significantly greater than normal in all three muscles examined. 2) Removal of the entire adrenal gland caused no significant alteration of glycogen in the leg and abdominal muscles, but in the diaphragm glycogen was elevated significantly. This latter value was, however, not as

great as that observed in the diaphragm of the adrenaldemedullated rat.

Hypophysectomized rats. 1) Removal of the pituitary severely lowered muscle glycogen. 2) Removal of the adrenal medulla produced glycogen values considerably above those of the animals which were hypophysectomized only. 3) Extirpation of the entire adrenal gland from hypophysectomized animals resulted in levels similar to, or slightly below, those found in hypophysectomized-adrenaldemedullated rats, and in all three muscles the glycogen values were well above those seen in the hypophysectomized controls. It can be observed that when all three influences, i.e., pituitary, adrenal cortex, and medulla were removed, the glycogen level of the diaphragm was the same as that of the normal animal.

TABLE 2. MUSCLE GLYCOGEN IN HYPOPHYSECTOMIZED AND HYPOPHYSECTOMIZED-ADRENALECTOMIZED MALE RATS

Treatment*	No. of animals	Rectus femoris	Abdominal muscle	Diaphragm muscle
		Glycogen (mg./100 gm. muscle)		
Hypophysectomized	5	316 ± 16†	396 ± 23	200 ± 16
Hypophysectomized-adrenalectomized	4	442 ± 57	528 ± 19	363 ± 17

* All values are from 24 hour fasted animals.
† Standard error of the mean.

In order to determine if sex of the animal played any part in the responses seen above, a group of 9 male rats were hypophysectomized and the adrenals subsequently removed from 4 of the animals. The results show, Table 2, that adrenalectomy of the male had a decided effect in relieving the low levels of muscle glycogen inflicted by hypophysectomy.

DISCUSSION

The influence of adrenalectomy on skeletal muscle glycogen has been somewhat questionable. Long *et al.* (1940) reported a fall in glycogen after adrenal removal, but did not specify which muscles were used. Illingworth and Russell (1951) found that of two skeletal muscles examined each behaved differently after adrenalectomy. The gastrocnemius showed no effect and the diaphragm glycogen was elevated. The present experiments confirm the latter work to the extent that the rectus femoris and abdominal muscle glycogen was unaffected by adrenalectomy, whereas the diaphragm contained greater amounts of glycogen.

Contrary to the results after adrenalectomy, all three muscles from adrenaldemedullated rats showed significantly greater amounts of glycogen than normal, and although the diaphragm level of the adrenalectomized rats was elevated, it was nevertheless below that of the adrenaldemedullated animals. The true effect of adrenal cortex removal on muscle

glycogen alteration probably should be considered as the difference between adrenaldemedullated levels and those found in the totally adrenalectomized animals. Upon this basis (Table 1) the cortex is seen to have a somewhat greater control than is sometimes implied. Removal of the adrenal medulla simultaneously with the cortex, as is done in adrenalectomy, masks the complete cortical effect, medullary absence tending to alleviate cortical deprivation. If it were possible to remove the cortex while retaining the medulla, glycogen levels would be expected to fall below those found in the totally adrenalectomized animal.

The fact, pointed out above, that the diaphragm showed an increase in glycogen above normal after adrenalectomy indicates that the medulla rather than the cortex may be the predominant adrenal factor in the control of glycogen in this muscle. Further indication of medullary influence over the diaphragm was seen in the hypophysectomized-demedullated rats, in which preparation diaphragm glycogen was equal to that of the normal intact animal. In other words, the low level of glycogen in the diaphragm of the hypophysectomized rat can be accounted for wholly, in these experiments, by unopposed medullary activity rather than absence of the pituitary, *per se*. This situation did not hold true for the other two muscles, in which glycogen was still somewhat below normal after adrenalmedullectomy of the hypophysectomized animals.

Total adrenalectomy of both male and female hypophysectomized rats gave increased glycogen levels in all muscles (Tables 1 and 2). From the other experiments presented, this result was presumably due to medullary, rather than cortical, deprivation.

The results of these experiments do not mean, necessarily, that muscle glycogen is at all times under the influence of the adrenal medulla. They do show that, when the medulla is present, a 24-hour period of starvation plus the excitement of handling and weighing previous to analysis are sufficient to lower muscle glycogen.

SUMMARY

Muscle glycogen levels were determined in fasting normal, adrenalectomized, and adrenaldemedullated rats, as well as in hypophysectomized, hypophysectomized-adrenalectomized and hypophysectomized-adrenaldemedullated animals. Removal of the adrenal medulla resulted in elevated glycogen levels in both normal and hypophysectomized rats. In animals with pituitary intact adrenalectomy exhibited no effect on glycogen of the rectus femoris and abdominal muscles, but gave higher values in the diaphragm. Adrenalectomy of hypophysectomized rats resulted in glycogen levels which, in all muscles, were above those found in animals merely hypophysectomized. Either adrenalectomy or demedullation of hypophysectomized animals gave diaphragm glycogen values equal to those of the normal intact rat.

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CYTOLOGICAL STUDIES ON THE HYPOPHYSECTOMIZED RAT ADRENAL CORTEX: THE ALTERATIONS OF ITS FINE STRUCTURE FOLLOWING ACTH ADMINISTRATION AND ON LOWERING THE Na/K RATIO[1]

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THIS work was undertaken as a sequel to an earlier electron microscopic investigation of the adrenal cortex in the normal rat (Lever, in press). The salient findings of this earlier study are as follows: (a) Throughout the cortex a subendothelial space separates the plasma membrane of the parenchymal and sinusoidal endothelial cells. (b) Cells light and dark in respect of electron opacity are found in all zones and may represent phases in a secretory cycle. (c) In light cells large numbers of thin-walled polyhedral sacs lie in juxtaposition to the mitochondria, some of which are vacuolated. (d) Dark cells are characterised by large numbers of mitochondria, of compact internal form, lying in a relatively opaque background substance. (e) An osmiophile substance is either elaborated by or accumulates within the mitochondria, and in all cortical zones, forms are discernible intermediate between mitochondria *per se* and densely osmiophile bodies which are probably lipid droplets.

The histology of the hypophysectomized rat adrenal has been widely investigated. Originally Smith (1930) reported that retrogression of the cortex was almost entirely responsible for the decrease in the rat adrenal weight following pituitary ablation. More selectively, Leblond and Nelson (1937) claimed that this cortical degeneration was most marked in the zonae fasciculata and reticularis. While Smith (1930) believed that a cytoplasmic reduction alone was responsible for cortical shrinkage, Crooke and Gilmour (1938) contended that there was also actual disappearance of cells in what they termed the "inner degenerative layers." According to them, the "outer cortical zone" (by which they probably meant the glomerulosa) not only was of normal appearance but actually increased in depth, in the absence of the pituitary. Swann (1940) and Deane and Greep

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(1946) argued that the zona glomerulosa might be the site of production of substances regulating sodium-potassium metabolism since this activity was minimally deranged after hypophysectomy. Deane, Shaw and Greep (1948) found that a lowering of the Na/K ratio in normal and hypophysectomised rats caused an increase in size and secretory activity of the zona glomerulosa. These workers are much inclined to the conception that the glomerulosa shows a high degree of independence of pituitary control in the rat and in the monkey (Knobil, Morse and Greep, 1955). This view has been questioned by Selye and Stone (1950) and Feldman (1951). They claimed that the entire cortex was under pituitary control, and that following hypophysectomy the glomerulosa became shrunken though not so markedly as the rest of the cortex. In the hypophysectomised rat adrenal, Lever (1955) observed that although the glomerulosa was initially wider than normal, yet it ultimately (after the 5th week) decreased in width because of cell shrinkage.

There is general agreement that replacement therapy by either anterior pituitary autografts or ACTH administration prevent all the changes resultant from hypophysectomy: or if they have occurred, such treatment restores the gland to a normal histological condition.

MATERIALS AND METHODS

Hypophysectomized male albino rats (white Wistar strain) of known preoperative weight were obtained from a commercial laboratory. Marked loss of body weight and an obviously diminished adrenal size (compared to the normal) were taken as evidence of effective hypophysectomy (Smith, 1930).

After removal, all glands were identically treated: each being divided into small representatively-layered fragments (less than 1 mm. thick) and immersed in Dalton's (1955) osmic-dichromate fixative for 50 min. After brief washing (10 mins.), specimens were rapidly dehydrated in ethyl alcohols and immersed, first in a 1:3 methyl-butyl methacrylate mixture (three changes) and then in a 0.2% solution of benzoyl peroxide in this mixture. Finally the tissues were transferred to capsules containing this methacrylate solution (partially polymerised for 3 hours at 37°C.) and hardened for 24 hours in a 47°C. oven. Sections in the order of 150Å were cut by glass knife on a modified Minot microtome and viewed with an RCA electron microscope (EMU. 2E). Gross orientation of the tissue was possible by phase microscopy of thicker sections (1μ) from the same block face.

The hypophysectomised adrenal was studied at 4 days (2 animals), 7 days (2), 10 days (2), 14 days (3) in one series; an opportunity of examining the gland 9 weeks after operation was provided by the control adrenals in the undermentioned experiments. *ACTH-stimulated rats.* Ten animals, hypophysectomised 9 weeks previously, were unilaterally adrenalectomised under ether anesthesia (thus providing control glands). The next day ACTH administration was started by intraperitoneal injection of the Armour "ACTHAR" gel preparation (20 units/cc.). In one group of 6 animals, each received 3 mgm. of ACTH/100 gm. body weight, twice a day (9 A.M. and 9 P.M.) and were killed singly by ether overdose at the following times after the commencement of treatment: 6 hours (after one injection), 24 hours (after two injections), 2 days, 3 days, 4 days and 7 days. In a second group the remaining 4 (hypophysectomised) rats, killed simultaneously at the 7th day, had received ACTH twice a day as follows: 1 mgm. (per 100 gm.

body weight), 2 mg., 3 mg. and 5 mg. respectively. In both these series, experimental glands were removed at the times specified and prepared for electron microscopy as described above. *Artificial depression of the Na/K ratio.* Unilateral adrenalectomy was performed under ether anesthesia in 6 rats hypophysectomised 9 weeks previously, the removed glands being used as controls. The next day potassium was administered by intraperitoneal injection of a 1% aqueous solution of KCl: a dose of 15 mg. KCl per 100 gm. body weight was given two-hourly for a total of 4 injections. Animals were killed at 9 hours (2 rats), 12 hours (2), and 36 hours (2) after the first dose, when the remaining adrenals were removed for electron microscopic investigation.

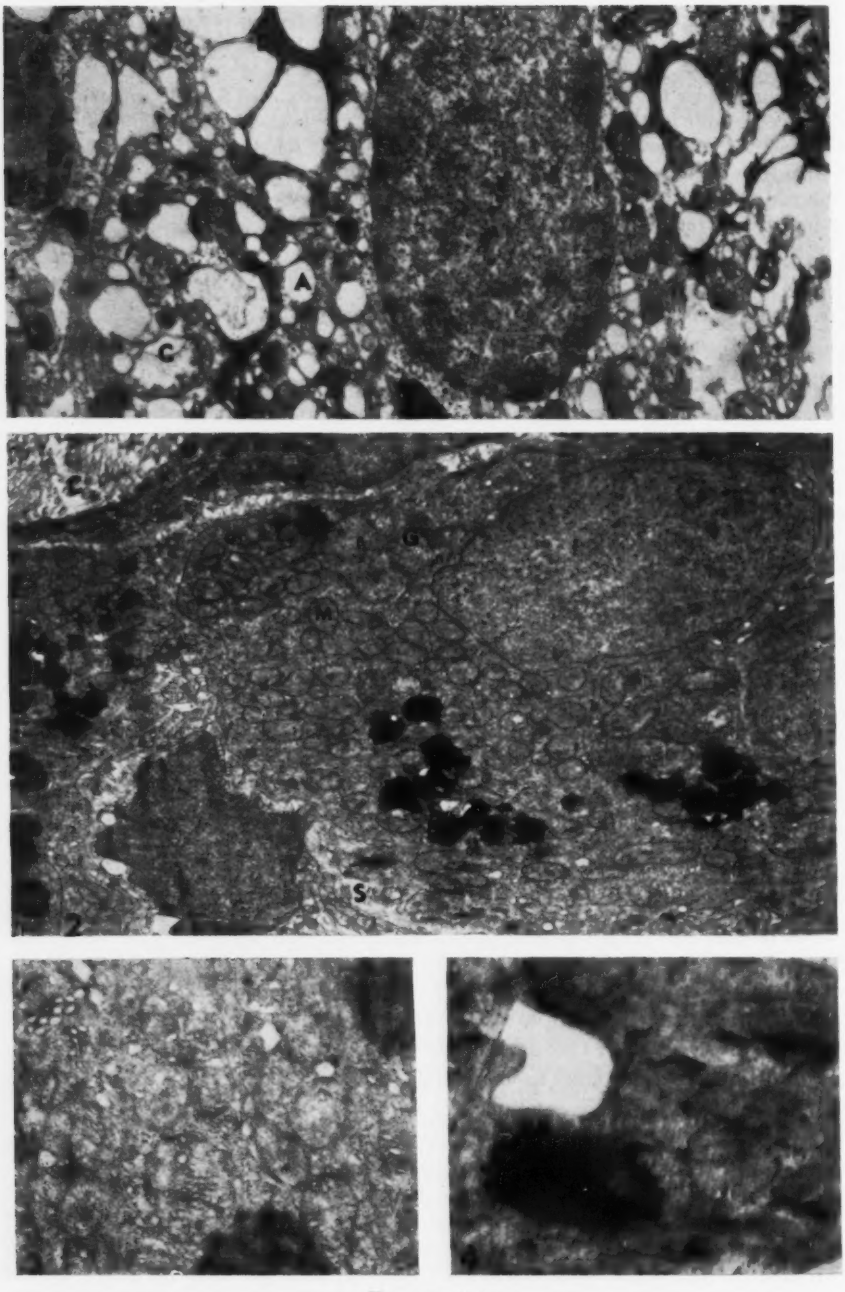
OBSERVATIONS

A. *Some effects of hypophysectomy on adrenocortical cytology*

Many of the changes in adrenal histology after hypophysectomy as observed by light microscopy, are amply confirmed at low magnification with the electron microscope. Observations of particular note are: (i) The integrity and apparent increase in depth of the z. glomerulosa for some time after operation. (ii) The prominence of the zona intermedia (Nicander, 1952; Lever 1954; Cater and Lever, 1954) which is synonymous with the "transitional" zone of Greep and Deane (1947) and the zone of "compression" of Mitchell (1948): this lies immediately deep to the z. glomerulosa. (iii) There is considerable difficulty in differentiating clearly between the zonae fasiculata and reticularis within the much shrunken inner cortex. (iv) A juxtamedullary cortical zone characterised by numbers of senescent cells, many of them patchily pigmented (Fig. 1), contains an inordinately large amount of collagenous tissue.

The Zona Gomerulosa. Fundamental alteration in cytology is not apparent (by electron microscopy) in the glomerulosa after hypophysectomy (Fig. 2) at any rate up to the 6th week. Reese and Moon (1938) reported "little or no" alteration in the form of the Golgi apparatus (by da Fano's technique) in the glomerulosa as compared with other zones following pituitary ablation. However, even after 7 days there is a noticeable crowding together of the cells within the zone and this is increased with time so that at the 6th and further at the 9th weeks, obvious transections of blood sinusoids are only a comparatively rare feature of a low power electron microscopic field as compared with the normal. This suggests a slow (much slower than in the other zones) cell shrinkage with resultant constriction of the sinusoids (Lever, 1954).

Although all the cell constituents as reported (Lever, in press) in the normal glomerulosa cell are easily recognizable 6 weeks after operation (Fig. 3), yet by the 9th week cytological detail becomes obscure and the sections exhibit only a dense blurred structure, (Fig. 4). Together with this decrease in cytological crispness, there is probably a diminished lipid elaboration or accumulation by the mitochondria since mitochondria heavily loaded with osmiophile material, as in the normal glomerulosa (Fig. 8), are rarely encountered. Furthermore it appears that with time



FIGURES 1–4

after hypophysectomy the outlines of mitochondrial and Golgi membranes become less electron opaque and hence less well defined.

The Zona Intermedia. A noticeable decrease in the cross sectional diameters of its cells (compared with those in the adjoining zones) in one of the most distinctive features of the zona intermedia at all times when it is clearly defined as a zone in the rat (Lever, 1954). Although frequently described as sudanophobe, in the normal and hypophysectomised rat adrenal (Reiss, Balint, Ostreicher and Aronson, 1936; Simpson, Evans and Li, 1943), yet in electron micrographs, it is commonly seen to contain irregular osmiophile aggregations. These are for the most part in close proximity to clusters of thin-walled sacs or vacuoles (Fig. 5) and the resulting appearances closely simulate those seen in certain cells of the zona reticularis and juxtamedullary cortex both in the hypophysectomized (Fig. 1) and in the normal animal (Lever, in press). In the zona intermedia there appears to be a reduction in the number of mitochondria per unit area of cytoplasm, relative to both the glomerulosa and fasiculata. These bodies have a loose irregular internum or are partly disintegrated and show no obvious evidence of lipid acquisition as judged by the absence of marked electron opacity. With time after hypophysectomy, as the zone broadens, its cells also become increasingly atrophic so that by the 9th week the majority of them are amorphous and compressed.

The Zonae Fasciculata and Reticularis. As already stated it becomes increasingly more difficult, with time after hypophysectomy, to make clear

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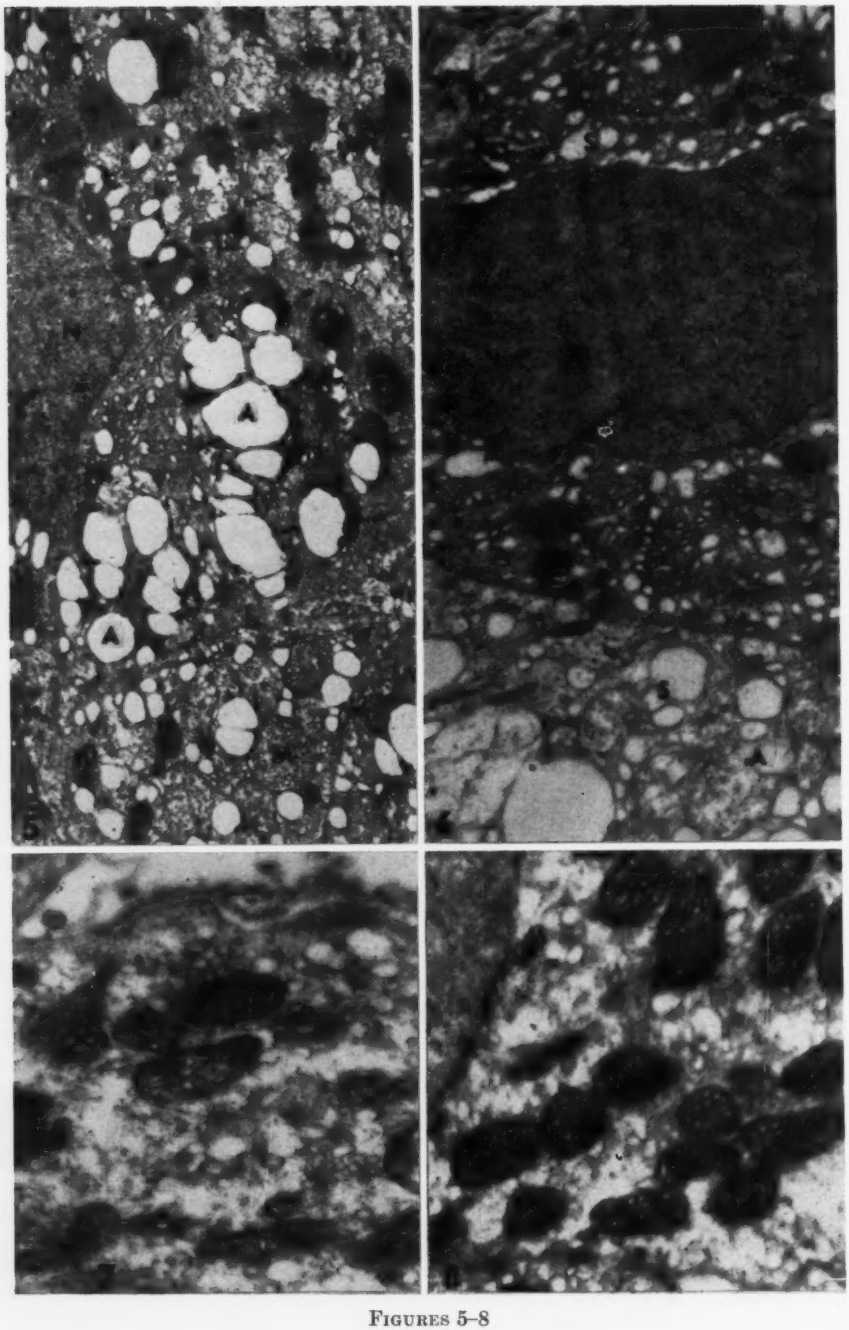
EXPLANATION OF FIGURES 1–4

FIG. 1. Section through the zona reticularis from a rat hypophysectomised two weeks previously. The reticularis cells are vacuolated, containing many thin walled, irregularly shaped sacs (A) which are contiguous to the dense, osmiophilic lipid substance (B) and to the moderately dense mitochondria. Some of the mitochondria are vacuolated (C). The cells of the reticularis frequently contain dense, granular bodies (D) which probably correspond to the intracellular pigment bodies characteristic of the zone. ×13,500.

FIG. 2. Section through the zona glomerulosa from a rat two weeks after hypophysectomy. Although the glomerulosal cells are somewhat smaller than those from control animals, their appearance is otherwise normal. The capsule of the gland appears at C, the subendothelial space separating the sinusoid from the parenchyma at S, mitochondria at M and the Golgi complex at T. The dense bodies are lipid droplets. ×9,000.

FIG. 3. Section through the zona glomerulosa six weeks after hypophysectomy. Lipid droplets still occur (lower center and upper right). Mitochondria are less dense than formerly (compare with Fig. 2) and the detail of their structure is less crisp. ×24,000;

FIG. 4. Section through the zona glomerulosa nine weeks after hypophysectomy. At this time the over-all density of the tissue is greater than normal and the detail visible in the mitochondria is almost completely obscured. It should be emphasised that this is a reproducible appearance and not merely an isolated, poor picture. ×24,000.



FIGURES 5–8

distinction between these two zones. Under these experimental conditions a cytological account is necessarily a description of change from the pre-existing normal electron microscopic pattern (Lever, in press) of these zones during their progressive degeneration.

The radially arranged cell columns which typify especially the mid fasciculata in the normal rat, are certainly still recognisable a week following hypophysectomy (Fig. 9), but after 2 weeks and more, this feature becomes obscured in the process of generalised cell shrinkage. The presence of both dark (electron opaque) and light cells in the fasciculata (earlier reported as a normal morphological feature in all zones of the adrenal: Lever, in press) can be confirmed in this material (Fig. 6) at any rate until the 6th post operative week. Because of the difficulty in defining the extent of a zona reticularis, it is perhaps safer to say that from an early stage (2 weeks after hypophysectomy) dark cells, which are a prominent feature of the normal reticularis, are rarely encountered in the immediately juxtamedullary cortex. The cells of this region closely resemble those in the zona intermedia (Figs. 1 and 5) in that they show a marked reaction (over the normal) in the amount of background material and in the number of mitochondria per unit area of cytoplasm, and in that they possess numerous clusters of thin-walled polyhedral sacs which lie in close proximity to irregular lipid aggregations: moreover many of the mitochondria are vacuolated. It appears that with time after hypophysectomy there is a centrifugal "expansion" in the extent of this juxtamedullary zone until at the 9th week it embraces all the cortex internal of the zona intermedia (which latter by then is ostensibly atrophic).

Evidence of the acquisition or elaboration of osmiophile material within

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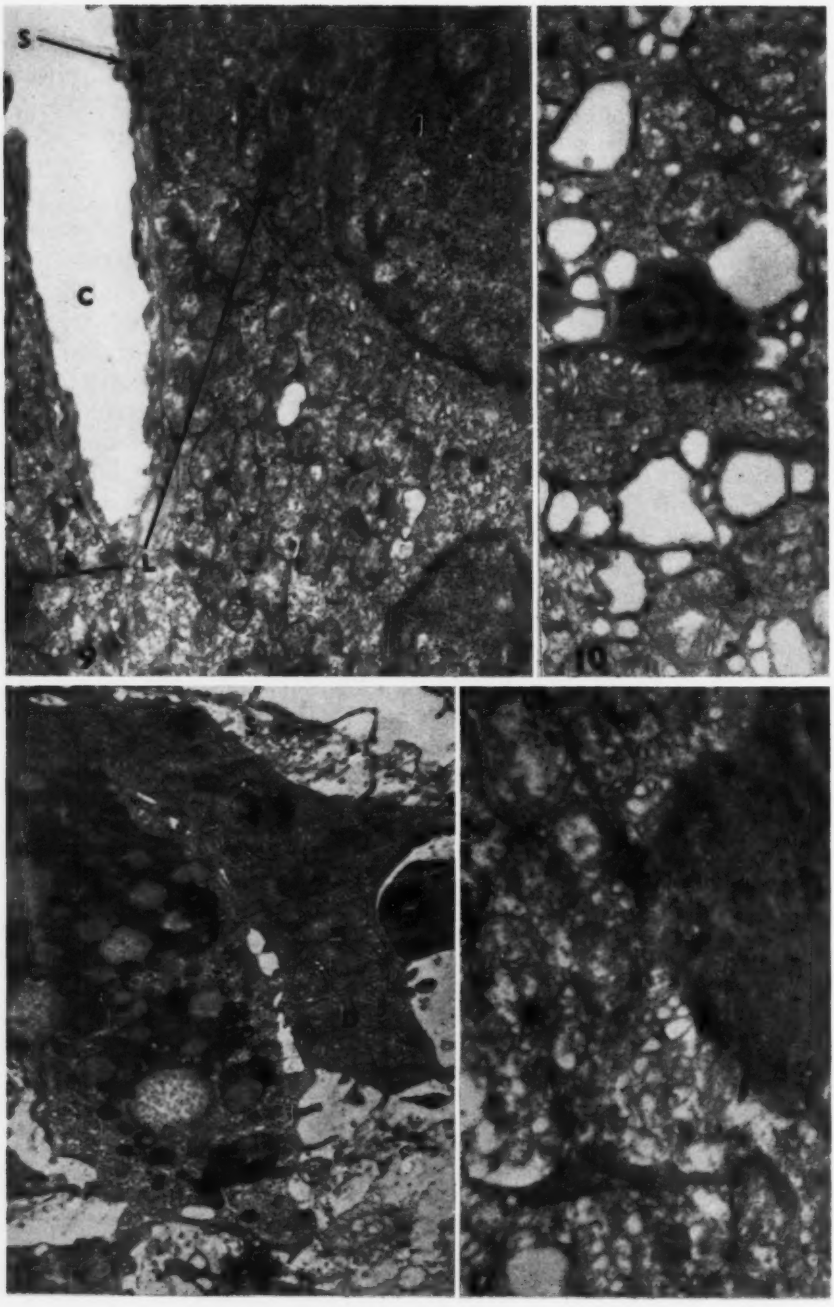
EXPLANATION OF FIGURES 5–8

FIG. 5. Section through the zona intermedia from a rat hypophysectomised two weeks. This zone consists of the flattened cells between the glomerulosa and the fasciculata which is especially noteworthy after hypophysectomy. It resembles the cells of the reticularis (compare with Fig. 1) and is distinct from the glomerulosa by virtue of the size of its cells. The reduced number of mitochondria (M) and by the large sacs (A) lying next to the lipid droplets (B). A nucleus is shown at (N). ×9,000.

FIG. 6. Section through the zona fasciculata two weeks after hypophysectomy. Parts of a "light" cell (below) and a "dark" cell (above) are shown. In the light cell, mitochondria have a loose internal structure and, in places, the outer membrane seems deficient so that the mitochondrion appears open as (A). (See also Fig. 16.) In the dark cell, the mitochondria are quite opaque and have a compact saccular internal structure. In both cells numerous thin-walled sacs are found (S). ×18,000.

FIGS. 7 and 8. Sections through the zona fasciculata from a rat hypophysectomised 4 days (Fig. 7) and a normal control (Fig. 8). Osmiophilia or opacity of the mitochondria is decreased in the hypophysectomised specimen. Fig. 7, ×25,000, Fig. 8, ×18,000.



FIGURES 9–12

the mitochondria can certainly be found in the zona fasciculata for at least 4 days after pituitary ablation (Fig. 7). Thereafter, direct evidence of this, in the form of a series of bodies ranging form mitochondria *per se* through intermediate forms to frank osmiophile droplets, has rarely been observed anywhere in the cortex. It must be added, however, that what appear to be droplets and irregular aggregations of lipid in variable (often very sparse) amounts remain a feature of all cortical cells right up to the time when (at about 9 weeks) nearly all cytological detail is lost. As in the zona glomerulosa, so in the fasciculata and reticularis, there is a progressive deterioration in the crispness of cytological detail after hypophysectomy (Fig. 12) and it becomes increasingly difficult to define the membranous internal structure of mitochondria or to identify with certainty the Golgi apparatus or the ergastoplasmic sacs: these changes are more marked in the fasciculata and reticularis than in the zona glomerulosa.

B. *Effects of a lowered Na/K ratio on the hypophysectomised rat adrenal cortex*

A very marked hyperplasia of the zona glomerulosa follows the lowering of the Na/K ratio of the rat 9 weeks after hypophysectomy: this change was not detected 6 hours after potassium administration but was well marked after 12 hours. As neither adrenal weights nor zone width measurements were made in this study it is not proven whether this regeneration involves a centripetal passage of gomerulosal cells replacing the underlying effete tissue, or whether the zone expansion is largely circumferential.

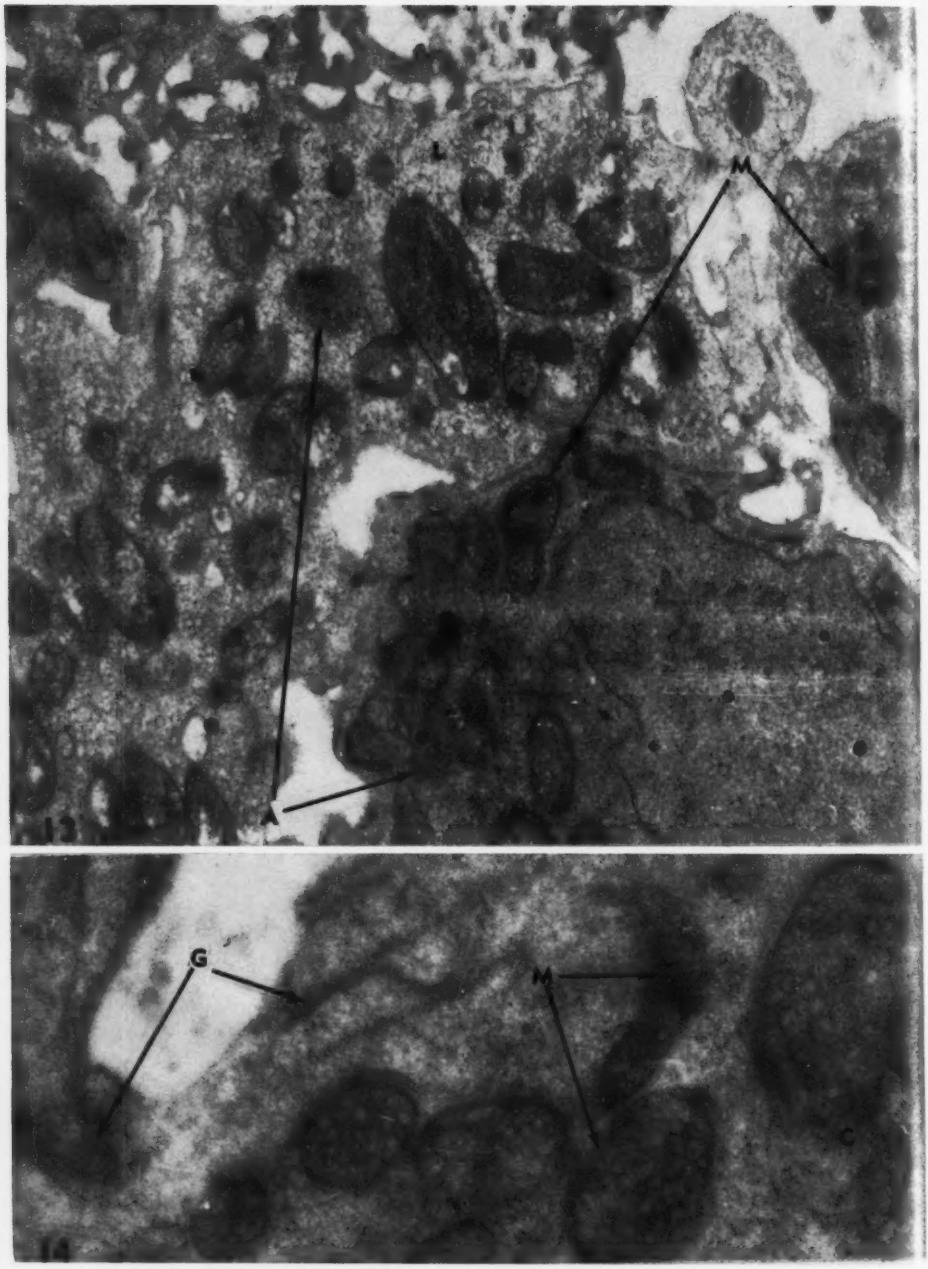
EXPLANATION OF FIGURES 9–12

FIG. 9. Section through the zona fasciculata one week after hypophysectomy. At this stage the cell columns are still quite dense, presumably because of their lipid content (L), but most of them now exhibit a loose, somehat ill-defined internal structure. The lumen of a sinusoid is shown at C, and the subendothelial space at S. ×13,500.

FIG. 10. Section through the glomerulosa from a rat, 9 weeks after hypophysectomy, which had been treated during the last 36 hours by lowering the Na/K ratio. Note the clusters of thin-walled sacs, and the appearance of the mitochondria, which contain filamentous and saccular internal membranes. ×27,000.

FIG. 11. Section from the juxta-medullary cortex of a rat nine weeks after hypophysectomy, which for 7 days had received 5 mg. ACTH twice daily. The "dark" cell (D) contains numerous, dense, compact mitochondria with crisp membranous outlines. Lipid is shown at (L). To the left is a cell containing large masses of a dense granular material which may be a lipochrome pigment. Note the wide, irregular subendothelial space (S) between parenchymal cell and sinusoid. ×13,500.

FIG. 12. Section from the outer fasciculata six weeks after hypophysectomy. In the dark cell at the right, detail is greatly obscured and, although it is less damaged in the light cell, the fine structure has hazy, rather than crisp outlines. Compare with Fig. 16. ×18,000.



FIGURES 13–14

Relevant to this is the observation that the degenerate or degenerating inner cortex in these treated glands appeared narrower than the controls; whether by compression or as a result of dissolution is not known. Furthermore there is an absence of a zona intermedia as described in the foregoing section, but a sharp delineation between the regenerating zone and the inner effete cortex is occasionally accentuated by cells (of glomerulosal derivation) containing pigmentous aggregations probably within their mitochondria.

Within this hyperplastic glomerulosal zone, cytological detail is greatly improved over that in the untreated control gland (Fig. 13): there is sharper definition of plasma membranes, mitochondrial internal structure and Golgi microtubules. Furthermore there is again evidence which suggests that the mitochondria are the sites of accumulation or elaboration of an osmiophile material which is probably lipid (Fig. 13), as is the case in the normal adrenal. Light and dark cells already described as a normal appearance in all zones of the adrenal cortex are again a striking feature of this stimulated glomerulosal tissue (Fig. 13). In tissue, 12 hours after potassium treatment the light cell cytoplasm besides being less electron dense than that in neighbouring dark cells, contained variably vacuolated mitochondria and a few not very obvious microsacs. In this last respect the light cell at this stage more nearly simulates the corresponding cell in the embryonic adrenal (Lever, unpublished), rather than that in the normal adult gland.

Further features of this regenerating glomerulosal tissue which are primarily seen (in this series) in glands 12 hours after treatment, suggest a possible relationship between the Golgi microtubules and the mitochondria. In a number of instances it was noted that the external limiting

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EXPLANATION OF FIGURES 13–14

FIGS. 13, 14, and 15. After lowering the Na/K ratio, the cells of the zona glomerulosa hypertrophy. The cytological changes associated with this are here depicted. The sections are from animals hypophysectomised 9 weeks and then treated with potassium. The control, or untreated hypophysectomised glomerulosa is illustrated in Fig. 4 with which these figures should be compared.

FIG. 13. The "light" cell (L) cytoplasm contains small saccular bodies and moderately vacuolated mitochondria. In the dark cell the cytoplasm is more homogeneous and the mitochondria more compact. In several places (A) the outer membranes of the mitochondria appear to be deficient. At M, mitochondria are illustrated which are denser than most, presumably because of an increased lipid content. ×21,000.

FIG. 14. Within a dark cell tubules and saccules occur which resemble the membranes constituting the Golgi complex (G). These membranes are in places so closely associated with mitochondria that they are difficult to distinguish from its outer membrane (M). At C, the outer membrane of a mitochondrion appears deficient. ×45,000.

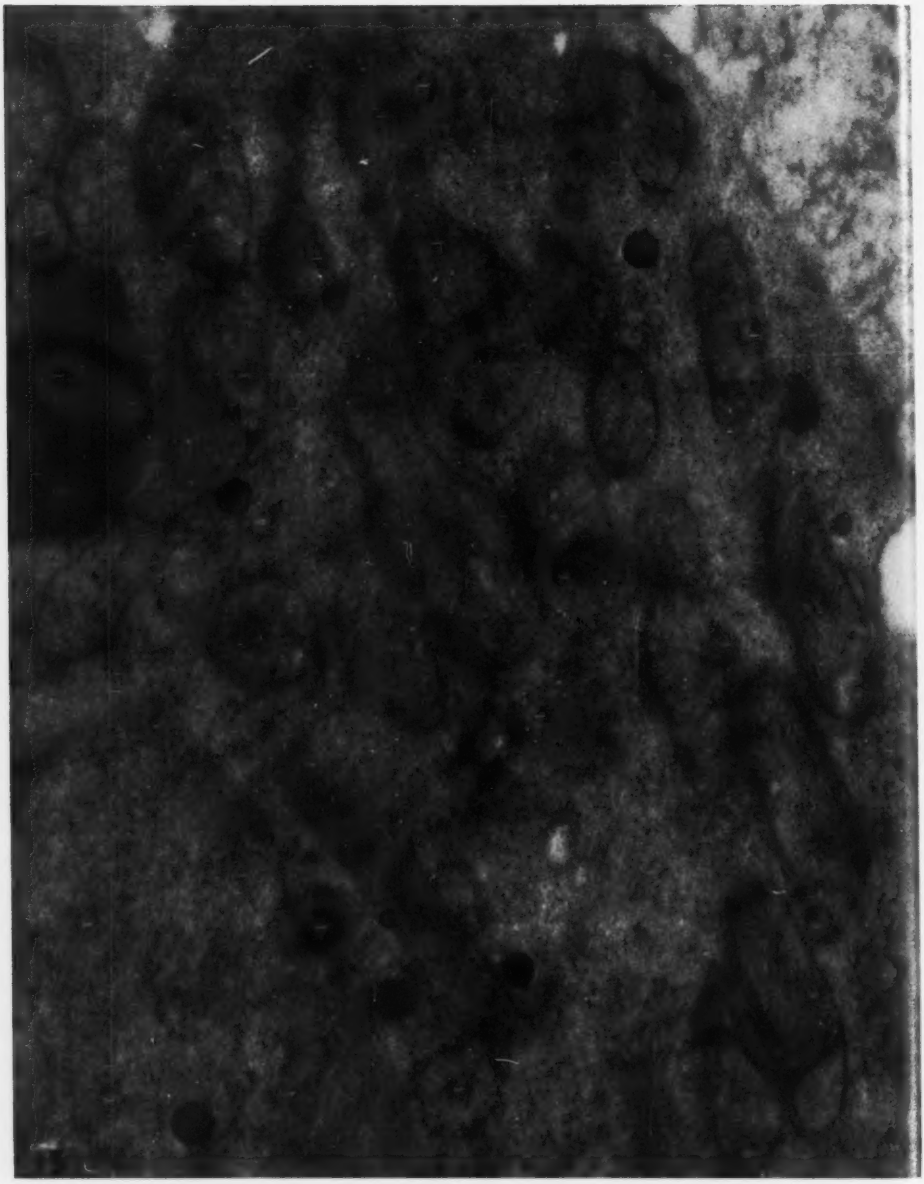


FIG. 15. Coplanar sheets of membranes or clusters of tubules of the Golgi type are illustrated at G. Within mitochondria similar sheets or clusters of membranes occur (M). ×45,000.

membrane of some mitochondria was deficient at one or more points, where their interna was in communication with the general cytoplasm (Fig. 14). Furthermore the mitochondrial limiting membrane is by no means always a uniform single or double line but may, in part of its extent, be represented by a series of near parallel lines (Fig. 15). In appearance these laminated membranes closely simulate the microtubules of the Golgi apparatus (Fig. 15). Indeed the internal membranous structure in many of the mitochondria in this hyperplastic tissue is in the form of a series of exceedingly fine microtubules indistinguishable in appearance from those of the Golgi apparatus except by their location. These observations are further supported in the following section on the ACTH-stimulated adrenal.

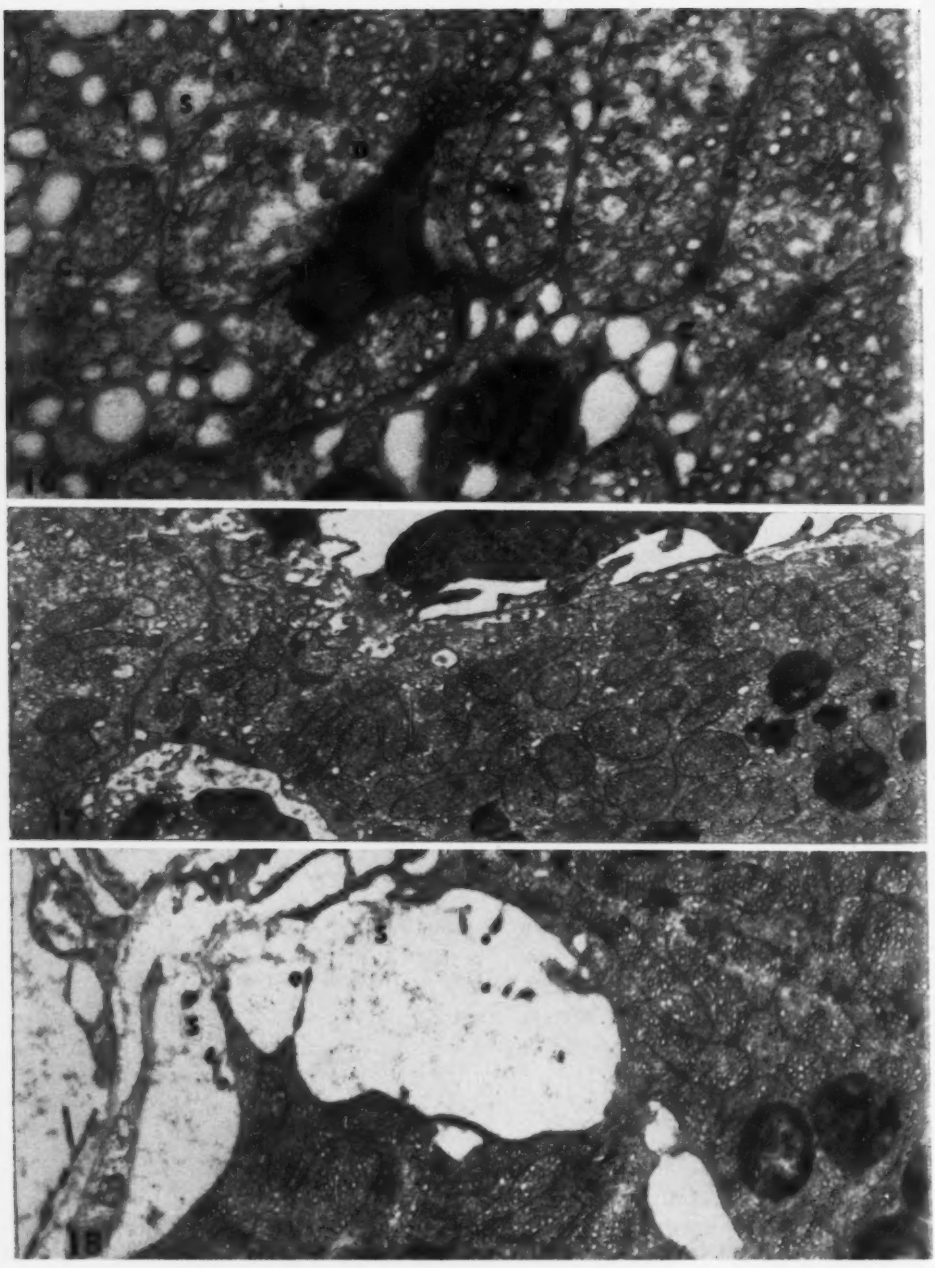
By the 36th hour after treatment the one-time regenerate zone is in the process of atrophy: its cells diminish in size and lose cytological detail and many of them have reverted to an appearance little different from the untreated control (hypophysectomised) adrenal. It is noteworthy that the light cells at this stage (Fig. 10), though sparse in number, possess many polyhedral cytoplasmic sacs and thus more nearly simulate those seen in the normal adrenal than do the light cells previously described in the tissue 12 hours after potassium administration (V. S.).

C. *The hypophysectomized rat adrenal after ACTH treatment*

It was found that the adrenal 9 weeks after hypophysectomy was not very plastic to ACTH except at high and prolonged dosage levels. Thus the best gland-restoration was obtained after a 7-day period on dosages of 3–5 mg. ACTH per 100 gm. body weight twice daily.

Unlike the regeneration of the zona glomerulosa following a lowered Na/K ratio which is limited and peripheral, restoration of the complete thickness of the cortex is the sequel to adequate ACTH replacement therapy, as was earlier shown by Reiss *et al.* (1936) and by other workers. The gross histological arrangement of the restored gland is not so regular as in the normal but the three main zones can be identified; and in particular the zona fasciculata because of its radially arranged cell columns (Fig. 17). In many places the parenchymal cells seem widely separated from the endothelia of their regional blood sinusoids by an irregular and extensive subendothelial space. This poses the question of whether the regenerating tissue is accompanied by a regenerating capillary system or whether it merely fits into the spaces between pre-existing sinusoids: no information has been obtained on this matter.

Cytologically, only slight differences distinguish the ACTH-treated gland from the normal. Thus (as in the normal), evidence that the mitochondria are the sites of lipid accumulation or elaboration can be found in all the reconstituted zones (Figs. 18 and 16). There is an increase (probably in excess of normal) in the number of mitochondria per unit area of



FIGURES 16–18

cytoplasm of all zones in the treated gland (Figs. 17 and 18). Miller (1953) has observed "mitochondrial proliferation" by light microscopy after ACTH treatment of normal and hypophysectomized rats, but only in the zona fasciculata. There appear to be may more (than normal) pigment-containing cells in the juxtamedullary cortex is the treated gland (Fig. 11). Although supposedly actively secreting, the treated gland after 7 days does not contain a great deal of lipid in frankly deposited form. Possibly the tissue has been restored beyond the normal into a frankly hyperactive state.

Perhaps the most striking observation in these glands (restored by ACTH), relates to the appearance throughout the tissue, but mainly in the glomerulosal and immediately subglomerulosal cells, of certain of the mitochondria. As in the potassium-treated gland described above, the external limiting membranes of these mitochondria may be deficient at one or more points, whereat there is direct contact of their interna with the cytoplasm at large (Fig. 16). Moreover the mitochondrial external limiting membrane is not always a clearly defined single or bilaminar structure, and often consists of three or more ill defined layers.

DISCUSSION

Several cytological changes appear to be common to all zones of the rat adrenal cortex after hypophysectomy. With time after removal of the pituitary there is a progressive loss of crispness of outline and detail in the organelles; an absolute reduction in the number of mitochondria (least obvious in the glomerulosa); diminishing evidence of the accumulation or elaboration of osmiophile material in the mitochondria.

It has been possible by electron microscopy to discern that the cells of the zona intermedia have a similar cytology to that of the juxtamedullary

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EXPLANATION OF FIGURES 16–18

FIG. 16. Section through the zona fasciculata from an animal hypophysectomised 9 weeks and treated with 5 mgm. ACTH twice daily for seven days. At M is a dense body with internal structure, interpreted as a mitochondrion with lipid contained within its internal membranes. Some of the cytoplasmic saccules, such as that at S, have granular walls and therefore might be regarded as ergastoplasmic. Several mitochondria are illustrated in which the outer membranes are deficient at C, so that their interior communicates with the surrounding cytoplasm. ×36,000.

FIG. 17. Section from the zona fasciculata of a rat treated as described in Fig. 16. Note the crispness of cytological detail as contrasted with the lack of definition exhibited in the untreated animal as illustrated in Figs. 9 and 12. X13,500.

FIG. 18. Section from the glomerulosa of a rat treated as described for Fig. 16. The figure should be compared with Figs. 3 and 4 to permit estimation of the reparatory effect of ACTH on the glomerulosa. Note the wide subendothelial space (S). Compact mitochondria are crowded together within the newly formed dark cells. ×22,500.

cells. They have few mitochondria, many of which are vacuolated with a loose disorganised membranous internum, and none of which show evidence of acquiring a content of osmiophile material. Athough commonly described as a subanophobe zone (in the rat), electron micrographs show the zona intermedia to contain what appear to be irregular lipid aggregates in close juxtaposition to numerous thin-walled sacs (Fig. 5). These facts and also the fact that it becomes cytologically amorphous before any other zone after hypophysectomy, strongly indicate that in this series the zona intermedia, whenever present, constitutes a region of heightened cell degeneration.

The irregular lipid aggregations which are a marked feature in the zona intermedia, the inner fasciculata and the reticularis of the short term hypophysectomised rat adrenal, have their counterpart in some of the reticularis light cells in the normal gland. It is significant that in all these situations lipid-containing mitochondria are few or absent. If this indicates a low lipid production in these zones, then the lipid aggregates are either: produced in some way other than within the mitochondria, or are previously produced within mitochondria the outlines of which have subsequently become obscured.

Pertinent to the correct interpretation of light and dark cells in the adrenal cortex, is the fact that in contrast to the normal, dark cells are rarely found in the inner fasciculata and reticularis of the hypophysectomised adrenal, which zones become markedly and rapidly degenerate. This would indicate that the adrenal dark cells may be young or formative, a concept which is supported by their content of numerous compact mitochondria.

The contention of Deane *et al.* (1948) that lowering of the Na/K ratio stimulates the zona glomerulosa is borne out by the present work. From an attenuated condition, the zone became hyperplastic and in its cells large numbers of mitochondria were produced and many of them were observed to be lipid-containing: but it must be emphasized that this tissue regeneration was localised, in the sense that the cells produced did not reconstitute the entire thickness of the cortex. On the other hand, ACTH administration leads to a complete cortical regeneration of all zones. Whether this regeneration is entirely from a glomerulosal cell source (Jones and Roby, 1954) or whether there is any reinstatement of existing atrophic cells deeper in the cortex, is unresolved. However it is clear from these facts that besides the quite definite electrolyte influence on the zona glomerulosa this zone is under a considerable degree of pituitary control.

Throughout this work, and in particular while studying the stimulated adrenal, the question of the mode of mitochondrial formation has been constantly in mind. It would seem that this organelle may lack a complete limiting membrane in certain circumstances. One of these is during its disintegration, as seen in many of the atrophic cells examined and as a

usual feature of the light cell in the normal zona reticularis. Again this is observed in cells known to be actively regenerating and in which there is rapid mitochondrial formation. In such a stimulated cell some of the mitochondrial membranes both limiting and internal may bear such a close resemblance to Golgi microtubules as to be indistinguishable from them (Fig. 15). It is perhaps profitable to speculate that in the stimulated rat adrenal, there is evidence which suggests a very close relationship between mitochondria and the Golgi apparatus.

SUMMARY

This paper describes an electron microscopic study of the hypophysectomized rat adrenal cortex and the changes produced in it following ACTH administration and also on lowering the Na/K ratio.

Following hypophysectomy there is reduction in the number and osmiophilia of the mitochondria together with a loss of cytological definition. These changes, progressive and common to all zones, are slighter and slower in rate in the zona glomerulosa.

The zona intermedia closely resembles the juxtamedullary cortical cells in electron micrographs and the likelihood of its being a zone of rapid cell degeneration is postulated. It contains aggregates of osmiophile material for some time after hypophysectomy.

Dark cells (which in the normal gland containing numerous mitochondria) are not a feature of the inner cortical zones after hypophysectomy. It is suggested that they may be young formative cells.

A temporarily lowered Na/K ratio results in a transient hyperplasia limited to the zona glomerulosa: there is an increase in the number of mitochondria, some of which contain lipid, and a clearer definition of all organelles in both light and dark cells. Similar cytological changes follow adequate ACTH treatment but these are more widespread since there is a full-thickness cortical restoration.

The integrity and activity of the zona glomerulosa would thus appear to be influenced both by the level of the Na/K ratio and by the anterior pituitary.

It would appear, in this experimental series, that the mitochondrion is not always a completely enclosed structure. In many cells, deficiencies in the limiting membranes of some mitochondria allowed continuity between their interna and the regional cytoplasm. The similarity in appearance between Golgi microtubules and the membranes composing some of the mitochondria suggests a close relationship between these two organelles.

Acknowledgments

It is a pleasure to acknowledge the kindly advice and criticism of Dr. E. W. Dempsey and to express my thanks to Mrs. Dorothy Goldstein for her technical help at the animal operations.

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EFFECT OF THYROID ACTIVITY ON BRAIN FUNCTION AND BRAIN ELECTROLYTE DISTRIBUTION IN RATS[1]

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THE relationship between thyroid activity and brain function has long been recognized. Adequate thyroid secretion is necessary for normal differentiation of the nervous system. Athyreosis in children results in marked mental retardation, and hypothyroidism in adults is characterized by mental sluggishness. On the other hand, patients with hyperthyroidism show symptoms of increased excitability of the nervous system. Similarly, thyroidectomy, hypothyroidism and thyroxine administration produce predictable alterations in brain excitability in laboratory animals. Because of space limitations, the numerous publications concerned with the influence of the thyroid gland on the central nervous system will not be discussed here; the reader is referred to recent articles on the subject (Woodbury *et al.*, 1952; Domino and Minz, 1953; Woodbury, 1954; Condon *et al.*, 1954).

In view of the practical importance of neurological symptoms in thyroid diseases and because of the frequent use of thyroid hormone in therapy, it was deemed of interest to study experimentally the effects of various thyroidal states on the brain function and to investigate their possible mechanism(s). In the present study, the effects of thyroidectomy, hypothyroidism, and thyroxine and triiodothyronine administration on brain function in rats were correlated with changes in body temperature, basal metabolism and brain electrolyte distribution. In addition, the influence of the adrenal gland on the effects of thyroid hormones on brain function and electrolyte metabolism was investigated.

MATERIALS AND METHODS

Sprague-Dawley male rats of an average initial body weight of 250 gm. were used in all experiments. Four experiments were performed:

In *Experiment I*, 40 rats were divided into 4 equal groups: 1) untreated controls; 2) thyroidectomized; 3) propylthiouracil-treated; 4) thyroidectomized and propylthiouracil-treated. Propylthiouracil (0.2%) was incorporated in the food (Calf Meal Builder), the daily dose ingested being that which is ordinarily employed to produce hypothyroidism. All animals were sacrificed on the 40th day of the experiment. In groups 1 and 2,

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blood was collected and brain (cerebral cortex) was removed for water and electrolyte analyses.

In *Experiment II*, 90 rats were divided into 9 equal groups: 1) untreated controls; 2) and 3) thyroxine-treated, each receiving 25 and 100 μg. daily, respectively; 4) and 5) triiodothyronine-treated, each receiving 5 and 20 μg. daily, respectively; 6) dinitrophenol-treated; 7) adrenalectomized; 8) adrenalectomized, thyroxine-treated (100 μg. daily, per animal); 9) adrenalectomized, triiodothyronine-treated (20 μg. daily, per animal). Dinitrophenol was incorporated in the food so that the daily intake was 20 mg. per kg. of body weight. All animals were sacrificed on the 14th day of the hormone treatment. Blood was collected and brain (cerebral cortex) was removed for water and electrolyte analyses.

In Experiments I and II, the alternating current electroshock seizure threshold (EST) test was employed to measure brain excitability (see below).

In *Experiment III*, 126 rats were divided into 3 groups of 30 (group 1) and 48 (groups 2 and 3) animals each. The experiment was divided into 2 parts: In the first part, the effects of 3 different doses of thyroxine (100, 200 and 400 μg. per animal) and of triiodothyronine (20, 40 and 80 μg. per animal) on the maximal electroshock seizure (MES) pattern were investigated. All animals in groups 2 and 3 were tested at intervals of one week. They were shocked at 30 minutes and at 1, 2, 4, 8, and 24 hours after the injection of thyroxine (group 2) or triiodothyronine (group 3). The values obtained were compared with those of the controls (group 1). In the second part, the postictal recovery time (see below) was studied in control animals and in thyroxine- (200 μg. per animal) and triiodothyronine- (40 μg. per animal) treated rats. The doses employed were those most effective in modifying MES pattern in the first part of the experiment. The time interval between the injection and electroshock was also that found to be optimal in the first part of the experiment, namely, 8 hours for thyroxine, and 4 hours for triiodothyronine.

In *Experiment IV*, 32 rats were divided into 4 equal groups, arranged as follows: 1) untreated controls; 2) thyroxine-treated (100 μg. daily, per animal); 3) propylthiouracil-treated; 4) thyroidectomized. All animals were sacrificed on the 16th day of treatment. Maximal electroshock seizure (MES) pattern and postictal recovery time were used as measures of brain activity.

All intact and thyroidectomized animals were maintained on an adequate diet and allowed free access to food and water. All adrenalectomized rats received 0.9% NaCl solution as drinking fluid.

The completeness of thyroidectomy was tested by measuring the 24-hour I^{131} uptake; that of adrenalectomy, by careful examination of the suprarenal region at autopsy.

Thyroxine[2] and Triiodothyronine[3] were dissolved in 0.9% NaCl solution to which had been added two drops of 2.5N NaOH. The solutions were injected subcutaneously. In the chronic experiments, the total daily dose was injected in two equal portions, at 9 A.M. and 5 P.M.; in the acute experiments, as a single dose.

Body temperature, measured rectally, was recorded at various intervals in Experiments I and II. The measurement was made at the same hour of the day (between 3 and 4 P.M.), to avoid interference from diurnal variations (Halburg *et al.*, 1954).

Basal metabolic rate (BMR) was determined with a constant-pressure-type respirometer.

Electroshock technics and apparatus employed in these experiments have been described elsewhere (Swinyard and Toman, 1948; Swinyard *et al.*, 1952; Woodbury and Davenport, 1952).

In determination of thresholds for minimal electroshock seizures (EST test), the

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[2] Thyroxine was generously supplied by M. N. Donin, E. R. Squibb & Sons.

[3] Triiodothyronine was obtained through the courtesy of Smith, Kline & French Laboratories, via Glaxo Laboratories where it was synthesized.

animals were tested until stabilization occurred. Thyroidectomy and adrenalectomy were performed 24 hours after threshold stabilization, and the animals were shocked again 24 hours after the surgical procedure. Thyroxine, triiodothyronine, propylthiouracil and dinitrophenol treatments were then started, and the EST was determined 3 times weekly in each rat. The changes in threshold during the treatment period were expressed as a percentage of the pre-treatment threshold. Maximal electroshock seizures (MES) are characterized by generalized tonic-clonic convulsions. Total duration of the seizure, duration of the tonic phase (flexor and extensor components) and duration of the clonus were determined for each animal. The values obtained in the treated animals were expressed as percentage of those recorded in control animals.

The recovery time (RT_{50}) was defined as the time required, after an initial maximal electroshock seizure, for 50% of the animals to recover to the extent that a second maximal seizure could be elicited. The data obtained from control and treated animals were plotted on probit paper, and RT_{50} and 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (1949).

Water and electrolyte analyses were performed by the methods in use in this laboratory and described in detail in previous publications (Woodbury, 1953; Timiras *et al.*, 1954).

RESULTS

1) *Comparative effects of thyroidectomy, propylthiouracil, thyroxine, triiodothyronine and dinitrophenol on brain function*

Brain excitability, as measured by the EST test, did not vary significantly in control animals throughout the experiment (Figs. 1 and 2).

Brain excitability was markedly decreased after thyroidectomy (Fig. 1). The lowered brain excitability was preceded, in the first days following thyroidectomy, by a transient increase, probably owing to the operative trauma. Propylthiouracil decreased brain excitability, as indicated by a rise in EST. By the 40th day, the EST values of the thyroidectomized and propylthiouracil-treated animals had reached a maximum of +25%, a value significantly different from that of the controls. When propylthiouracil was administered to thyroidectomized animals, the increase in threshold was significantly greater than in the intact animals and reached a peak value of +40%.

Small doses of thyroxine (25 μg.) did not appreciably modify brain excitability until the 9th day of the experiment (Fig. 2). The EST then declined and was 8.5% below normal by the 14th day. After a 100 μg. dose of thyroxine, the EST values fell by 10% in the first three days of treatment. They steadily decreased to −23.5% by the 14th day, a value indicative of a marked increase in brain excitability. Triiodothyronine was administered in doses only one-fifth as large as those of thyroxine and yet the decrease in seizure threshold produced by both hormones was similar; 5 μg. of triiodothyronine reduced EST by 11% and 20 μg. reduced EST by 23%, by the 14th day of treatment (Fig. 2).

Dinitrophenol did not modify brain excitability (Fig. 2).

The effects of a single injection of thyroxine and triiodothyronine on the pattern of maximal electroshock seizures (MES) are presented in Figure 3. This type of tonic-clonic seizure is constant in severity and in the sequence

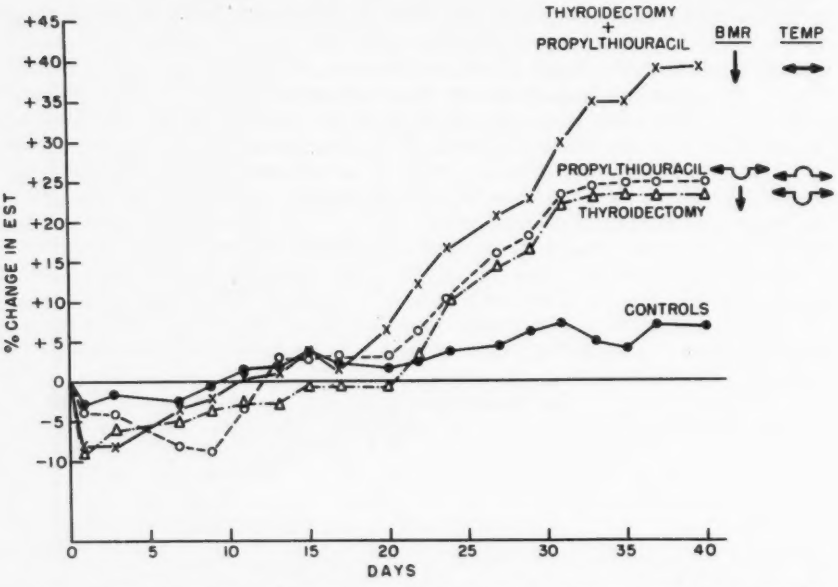

FIG. 1. Experiment 1. Effect of thyroidectomy and propylthiouracil, alone and in combination, on electroshock seizure threshold (EST) in rats.

Ordinate is per cent change in EST referred to control values at zero time. Abscissa is time in days. Values for untreated controls are shown for comparison.

Arrows shown to the right of the EST curves represent changes in basal metabolic rate (BMR) and in body temperature in the different groups as compared with the untreated controls. The relative length of the shaft of the vertical arrows indicates the relative intensity of the change in the response listed; the direction of the vertical arrows indicates the direction of the change in response. Horizontal arrows indicate that there is no significant change in response; a tendency of the response to increase or decrease is shown by the appropriate curve in the shaft of the horizontal arrows.

and the duration of its components. After application of the current (0.2 sec.), a brief latency period is followed by a tonic phase of the seizure which consists of flexion followed by hindleg extension; the subsequent purely clonic phase is followed by abrupt relaxation and postictal depression. Prolongation of flexion and shortening of extension in the tonic phase of the seizure are indicative of an anticonvulsant effect and/or decreased brain excitability; the ratio of flexion to extension duration is decreased in states of increased excitability. The values in Figure 3 represent those obtained at the time of peak effect of a single dose of the two hormones. Both thyroxine and triiodothyronine significantly prolonged the tonic extensor phase and shortened the tonic flexor and clonic phases. The changes in MES pattern were more marked at the higher dose levels; again triiodothyronine exerted the same effect on MES as did thyroxine, but its effective dose was only one-fifth that of thyroxine. In chronic experiments, the effects of thyroxine on MES were similar to those described after a single dose (Fig. 4).

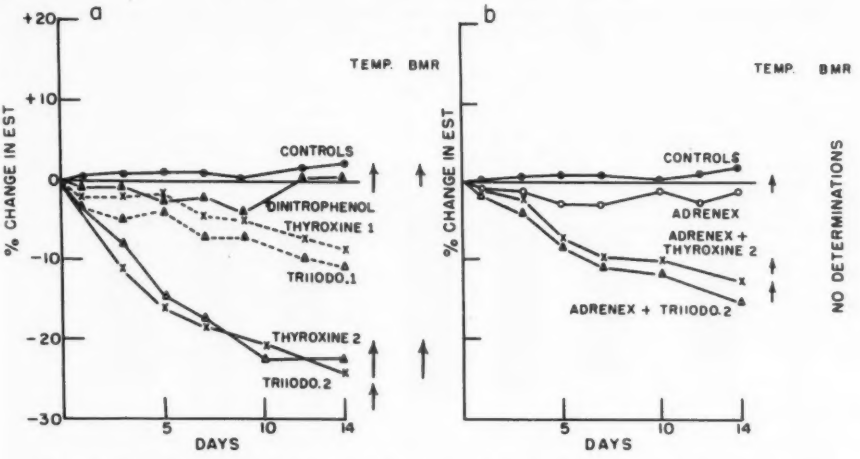

FIG. 2. Experiment II. *a*. Effect of thyroxine, triiodothyronine and dinitrophenol on electroshock seizure threshold (EST) in *intact* rats.

Thyroxine (1) and thyroxine (2), 25 and 100 μg. daily per animal, respectively; triiodothyronine (1) and triiodothyronine (2), 5 and 20 μg. daily per animal, respectively.

b. Effect of thyroxine and triiodothyronine on electroshock seizure threshold (EST) in *adrenalectomized* rats.

Thyroxine (2), 100 μg. triiodothyronine (2) 20 μg., daily per animal.

Ordinate is per cent change in EST referred to control values at zero time. Abscissa is time in days. Values for untreated and adrenalectomized controls are shown for comparison.

Arrows at the right of the EST curves represent changes in body temperature and in basal metabolic rate (BMR) (see Fig. 1).

Propylthiouracil prolonged significantly the duration of the tonic flexor component, thus increasing the total duration of the seizure. Thyroidectomy produced an effect opposite to that of propylthiouracil, namely, a marked shortening of the flexor and a marked prolongation of the extensor component. In addition, the total duration of the seizure was significantly longer than in the controls (Fig. 4).

The effects of thyroidectomy, propylthiouracil, thyroxine and triiodothyronine on postictal recovery time (RT_{50}), in acute and chronic experiments, are presented in Figure 5. No significant changes in postictal recovery were produced by a single injection of thyroxine or triiodothyronine. When given chronically, thyroxine produced a slight acceleration and thyroidectomy a marked acceleration of recovery, whereas propylthiouracil prolonged the duration of postictal depression.

2) *Comparative effects of thyroidectomy, propylthiouracil, thyroxine, triiodothyronine and dinitrophenol on body temperature, basal metabolic rate and brain electrolyte distribution*

The values for body temperature and basal metabolic rate (BMR) measured at the end of Experiments I and II are indicated in Figures 1 and 2,

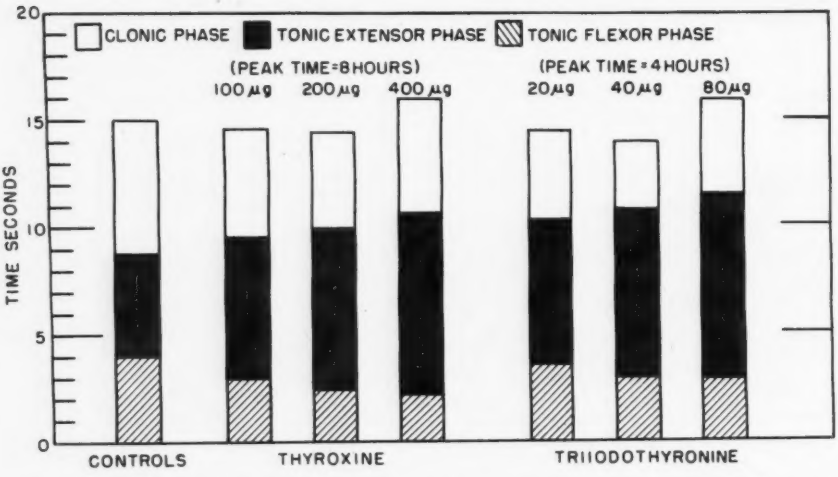

FIG. 3. Experiment III. Effect of three doses of thyroxine (100, 200 and 400 μg. per animal) and of triiodothyronine (20, 40 and 80 μg. per animal) on maximal electroshock seizure (MES) pattern in rats.

Each dose was given in a single injection and the MES pattern was determined at the time of peak effect (4 hours for triiodothyronine and 8 hours for thyroxine).

Ordinate is time in seconds; the various procedures are indicated on the abscissa. The pattern of seizures in the untreated controls is shown for comparison.

to the right of the EST curves. Thyroidectomy significantly decreased body temperature and BMR. Propylthiouracil decreased BMR but only slightly lowered body temperature. Thyroxine, triiodothyronine, and also dinitrophenol markedly raised body temperature and BMR.

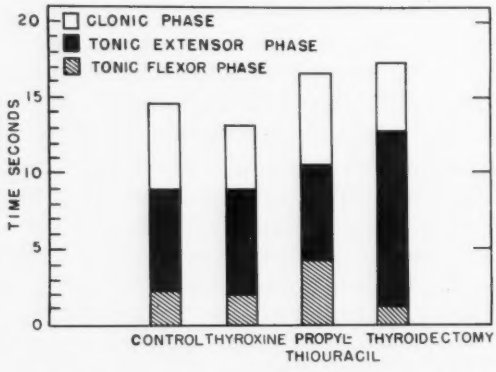

FIG. 4. Experiment IV. Effect of thyroxine, propylthiouracil and thyroidectomy on maximal electroshock seizure (MES) pattern in rats, as determined 16 days after beginning of treatment or after surgery.

Ordinate is time in seconds; the various procedures are indicated on the abscissa. The pattern of seizures in the untreated controls is shown for comparison

Figure 6 shows the effects of thyroidectomy, thyroxine and triiodothyronine on brain extracellular and intracellular Na concentration. Thyroidectomy increased extracellular and decreased intracellular Na concentration, and increased the ratio of extracellular to intracellular Na. It also decreased Na space. Thyroxine and triiodothyronine produced the opposite effects.

Brain intracellular and extracellular K concentration, Cl space and Cl distribution of plasma and brain were not significantly altered in any of the

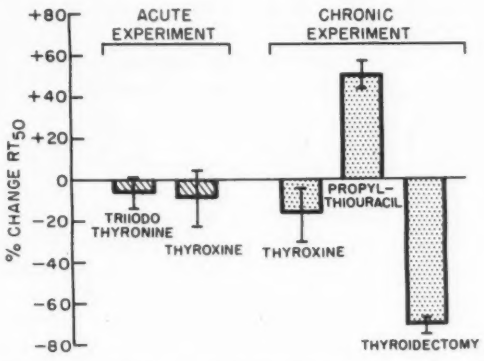

FIG. 5. Experiments III and IV. Effect of thyroidectomy, propylthiouracil, thyroxine and triiodothyronine, after single doses and after chronic administration, on postictal recovery time (RT_{50}).

Ordinate is per cent change in RT_{50} referred to values obtained in control animals. The various procedures and the duration of treatment are indicated on the abscissa. The 95 per cent confidence limits are shown by the vertical bracketed lines.

experimental conditions studied. The only exception was a decrease in the ratio of intracellular to extracellular K in thyroxine- and triiodothyronine-injected rats.

Dinitrophenol slightly decreased plasma and brain water but had no significant influence on Na, K and Cl concentrations of plasma or brain.

3) *Influence of the adrenal gland on the effects of thyroxine and triiodothyronine on brain excitability and electrolytes*

No significant changes in brain excitability and electrolyte distribution were observed in the adrenalectomized controls.

Thyroxine and triiodothyronine were less effective in increasing brain excitability and body temperature in adrenalectomized than in intact animals (Fig. 2). The lesser elevation in brain excitability was accompanied by modifications of brain electrolytes, and in particular of brain Na, which were qualitatively similar but quantitatively less marked than those in intact animals injected with thyroid hormones (Fig. 6).

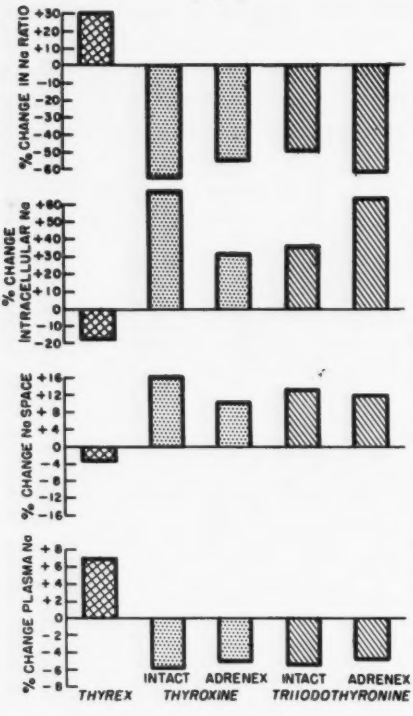

FIG. 6. Experiments I and II. Effect of thyroidectomy, and of thyroxine and triiodothyronine on Na metabolism of cerebral cortex and plasma, in intact and adrenalectomized rats.

Ordinates, as shown in figure from below upward, refer to per cent change in plasma Na concentration, brain Na space, intracellular brain Na concentration (calculated by use of chloride space as a measure of extracellular fluid volume of the brain), and ratio of extracellular to intracellular brain Na concentration.

Per cent changes are calculated by use of the intact and adrenalectomized untreated animals as controls. The various procedures are indicated on the abscissa.

The changes in Na distribution, as well as in EST (Figs. 1 and 2), resulting from thyroidectomy are opposite to those produced by thyroxine and triiodothyronine. Adrenalectomy decreases the effects of the two thyroid hormones on Na distribution as well as on EST (Fig. 2).

DISCUSSION

The decrease in brain excitability after thyroidectomy and propylthiouracil and the marked increase in brain excitability after thyroxine and triiodothyronine are in agreement with the clinical reports, and confirm previous experimental observations from this laboratory (Woodbury *et al.*, 1952; Woodbury, 1954). Additional proof of the stimulatory action of thyroxine and triiodothyronine on brain function is provided by the observation that the two hormones, both after a single dose and after chronic administration, modify the pattern of maximal electroshock seizures and accelerate postictal recovery.

It should be emphasized that the stimulatory action of triiodothyronine on brain function resembles qualitatively that of thyroxine; however, in both chronic and acute experiments, triiodothyronine is five times more effective than thyroxine in its ability to increase brain excitability, the potency ratio of the two hormones being 1 to 5 on a weight basis.

The increase in brain excitability produced by thyroxine and triiodothyronine appears to be correlated with the dose administered; small doses lower seizure threshold values by about 10%; larger doses decrease threshold much more rapidly and produce a maximal decrease of approximately 23% below control levels.

In acute experiments, the time of peak effect is 8 hours for thyroxine and 4 hours for triiodothyronine. This indicates that triiodothyronine acts more rapidly on the brain than does thyroxine. However, the action of triiodothyronine is relatively transient; it disappears almost completely 8 hours after administration of the hormone, whereas thyroxine is still effective after 24 hours. This is in agreement with recent experiments showing that I^{131} labeled triiodothyronine penetrates into the brain more rapidly, but also leaves this organ at a faster rate, than does I^{131} labeled thyroxine (Vanarsdel *et al.*, 1954).

The markedly accelerated postictal recovery in the thyroidectomized rats is unexplained at the present time. One may speculate that these animals, because of their reduced BMR, have greater than normal cerebral stores of energy-rich phosphate compounds and hence are able to recover more rapidly. Before further speculation is warranted, the effect of thyroidectomy and thyroid hormones on brain phosphorous metabolism must be determined.

Since certain effects of propylthiouracil (modification of maximal electroshock seizure pattern and duration of postictal depression) differ from those of thyroidectomy, the possibility of a direct depressant effect on the central nervous system, in addition to an antithyroid action, must be considered. Since decrease in body temperature has been shown to increase brain excitability (Swinyard and Toman, 1948) the greater decrease in excitability induced by propylthiouracil as compared with thyroidectomy might also be explained by the lack of effect of propylthiouracil on body temperature as compared with the hypothermia induced by thyroidectomy (Fig. 1).

The increased brain activity which follows thyroxine administration was originally ascribed to an enhanced cerebral metabolism. A correlation was suggested, in normal subjects, between alpha frequency of the electroencephalogram and basal metabolic rate (Lindsley and Rubenstein, 1937). This interpretation, however, was challenged by Hall and Lindsay (1938) and Brody (1941) who advocated a direct influence of the thyroid hormone on the central nervous system. It was shown that, in hyperthyroid patients with very high metabolic rates (Sokoloff *et al.*, 1953) and in thyroidectomized rats injected with thyroxine (Barker and Klitgaard, 1952) the O_2 consumption of the brain remains normal even though the over-all O_2 consumption of the body rises markedly. Similarly, Hoexter (1954) showed that the O_2 uptake of brain cortex slices from thyroidectomized animals remained unchanged, while that of other tissues was decreased. Further proof of the dissociation between central nervous and general metabolic effects of thyroid activity is provided in the present investigation by the demonstration that dinitrophenol increases BMR but has no effect on brain excitability.

Previous experiments in this laboratory have shown that the changes

in electrolyte distribution produced by hormonal hyperfunction and hypofunction are associated with changes in brain excitability. Particular attention has been paid to the relation between adrenal cortex and electrolyte metabolism (Woodbury, 1954; Timiras *et al.*, 1954). In addition, our recent investigations (Woodbury, 1954; Timiras *et al.*, 1955) have suggested that hormones other than those secreted by the adrenal cortex may

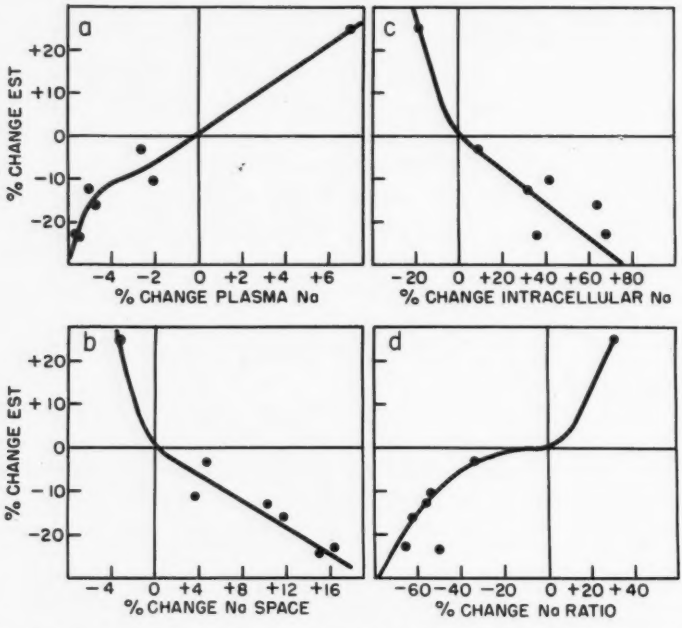

FIG. 7. Relation between brain excitability and Na metabolism in various thyroid states.

The experimental points are plotted from the values of the maximal changes in EST induced by thyroidectomy, thyroxine and triiodothyronine shown in Figures 1 and 2. The Na values are those summarized in Figure 6.

Ordinate is per cent change in brain excitability (measured by EST test). All per cent changes are referred to values obtained in control animals.

a. Relation between EST and plasma Na concentration. Abscissa is per cent change in plasma Na concentration.

b. Relation between EST and changes in Na space in brain. Abscissa is per cent change in brain Na space.

c. Relation between EST and intracellular brain Na concentration. (Intracellular brain Na concentration is calculated by use of the chloride space as a measure of extracellular fluid volume of the brain.) Abscissa is per cent change in intracellular brain Na concentration. Note similarity of this curve and that of figure 7b (Na space), an indication that the induced changes in Na space are probably due to intracellular movements of Na.

d. Relation between EST changes and variations in the ratio of extracellular to intracellular brain Na concentration. Abscissa is per cent change in this ratio.

also influence both brain excitability and electrolyte metabolism. Relatively few data on the effects of thyroid activity on electrolyte distribution in tissues and blood have been reported so far. Lundbaek (1947) has reviewed some of the biochemical changes which occur during thyrotoxicosis and its treatment; he concludes that thyroid hyperfunction is accompanied by changes in water and salt metabolism and that these changes revert to normal with the amelioration of the disease. The data presented in this investigation indicate that there is a close correlation between brain excitability and brain Na concentration (Fig. 7). Brain excitability appears to vary inversely with plasma Na concentration and directly with brain intracellular Na concentration and brain Na space, and inversely with the ratio of extracellular to intracellular Na concentration in the brain. It should be mentioned that, although electrolyte alterations appear to be important in affecting brain excitability, other factors (Woodbury, 1954) are probably also involved and further study is required to elucidate them.

The observation that thyroxine and triiodothyronine increase brain excitability and modify brain Na concentration significantly less in adrenalectomized rats than in intact animals suggests that part of the action of thyroid hormones on brain function and electrolyte metabolism is mediated through the adrenals. It can be assumed, therefore, that thyroxine and triiodothyronine increase brain excitability by a dual mechanism: a direct stimulation of the central nervous system and an indirect stimulation via an increased secretion of adrenocortical and/or adrenomedullary hormones. This hypothesis is further corroborated by recent observations which indicate that thyroid hormones increase adrenocortical function (Eartley and Leblond, 1954) and which describe the effects of adrenocortical (Woodbury, 1954; Timiras *et al.*, 1954) and adrenomedullary (Minz and Domino, 1953) hormones on brain function.

SUMMARY

The effects of thyroidectomy, propylthiouracil, thyroxine and triiodothyronine on brain function and brain electrolyte metabolism were studied in rats.

The most important results can be listed as follows:

1) Thyroidectomy decreases brain excitability. However, the capacity of the brain to sustain maximal activity is retained or possibly increased.

2) Propylthiouracil decreases brain excitability. It also decreases the capacity of brain to sustain a maximal electroshock seizure, and prolongs postictal depression.

3) Thyroxine and triiodothyronine increase brain excitability, enhance the capacity of the brain to support maximal electroshock seizures, and shorten postictal depression. The relative stimulatory potency of thyroxine and triiodothyronine on brain function is 1 to 5. For both hormones, the

stimulatory effect on brain activity is directly related to dose and duration of treatment.

4) Dinitrophenol, administered in doses sufficient to produce a marked increase in basal metabolic rate, does not modify brain excitability and electrolyte metabolism.

5) Concomitantly with brain excitability variations, thyroidectomy, thyroxine and triiodothyronine produce alterations in brain electrolyte metabolism, the most important of which are those concerned with Na distribution.

6) Stimulation of the central nervous system and alterations in brain electrolyte distribution induced by thyroxine and triiodothyronine are less marked in adrenalectomized than in intact rats. This suggests that the thyroid hormones stimulate brain function and alter brain electrolyte distribution both by a direct action and through stimulation of the adrenals.

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INFLUENCE OF SOMATOTROPHIN AND CORTISONE ON THE INSULIN SENSITIVITY OF RATS[1,2]

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INSULIN sensitivity, i.e., the magnitude of the hypoglycemic response to a test dose of insulin, is influenced by a multitude of factors, including other hormones. Since the discovery of Houssay and Magenta (1925) and Lewis (1923) that hypophysectomized or adrenalectomized animals are abnormally sensitive to insulin, much evidence has accumulated regarding the identity of the hormones which affect insulin sensitivity. According to current views, the most important of these are the adrenal glucocorticoids and somatotrophin (de Bodo and Sinkoff, 1953a), both of which have been repeatedly shown to possess anti-insulin activity.[3]

The most systematic studies of endocrine factors that affect insulin sensitivity (de Bodo and Sinkoff, 1953a and b, de Bodo and Altszuler, 1955) were performed on dogs and comparably exhaustive studies on rats have not been published. The present paper deals with the effects of STH and cortisone separately and in combination on the insulin sensitivity of intact, hypophysectomized, adrenalectomized and hypophysectomized-adrenalectomized rats.

MATERIALS AND METHODS

Young adult male rats of the Sprague-Dawley strain, weighing between 150 and 350 gm., were used in this study.

Intact rats were maintained on Rockland pellets *ad libitum* and tap water.

Bilateral adrenalectomy, under nembutal anesthesia, was performed in one stage through a single dorsal incision. After the operation the animals were allowed Rockland pellets *ad libitum* and received 0.9% sodium chloride in their drinking water. Their insulin sensitivity was determined on the fifth postoperative day.

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[2] Based on a dissertation submitted by Dr. Spirtos in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Anatomy of the Graduate College of the State University of Iowa, June, 1955.

[3] The observations of Westermeyer and Raben (1954) suggest that the pituitary factor which antagonizes insulin without the mediation of glucocorticoids may actually be a potent smaller molecule which is present in ACTH as well as STH preparations. Whenever the effects of STH are referred to in this paper, this is done with the mental reservation that the moiety which affects carbohydrate metabolism may not be identical with that which is protein anabolic. Furthermore, the terms "insulin antagonism," "anti-insulin action" and "synergism" of hormones will be used with blood sugar changes as the sole frame of reference. The metabolic loci at which insulin and its pituitary or adrenal "antagonists" act may be altogether different.

Hypophysectomized rats, obtained from the Hormone Assay Laboratories of Chicago, were fed Rockland pellets *ad libitum* supplemented with horse meat and oranges, and received tap water. They were used for the determination of insulin sensitivity 6 to 22 days after the operation. The length of the postoperative interval did not appear to influence the response to insulin.

Groups of hypophysectomized animals were adrenalectomized under nembutal anesthesia on the eighth day after the removal of the hypophysis. In control hypophysectomized rats the adrenals were exposed but not removed. Both hypophysectomized-adrenalectomized rats and their hypophysectomized-sham adrenalectomized controls were continued on the supplemented pellet diet but were given physiological saline instead of tap water. Insulin sensitivity determinations on these animals were performed on the fifth day after adrenalectomy or the sham operation.

When somatotrophin (STH)[4] and/or cortisone[5] were administered, they were injected subcutaneously once daily for a period of 5 days, with the last injection given 2 hours prior to the injection of insulin. The usual dose was 0.5 mg. Armour standard equivalent of STH and/or 0.5 mg. of cortisone daily per 100 gm. of body weight. These will be referred to as the standard dosages. In some experiments 0.1 of the standard dose for either substance was given over the same period of time and following the same schedule.

All animals were fasted for 24 hours prior to the determination of insulin sensitivity, which was carried out by recording the blood sugar response to an intraperitoneal dose of 0.1 unit of insulin[6] per kg. of body weight. A number of intact rats were given one unit per kg. Blood samples (0.1 ml.) were taken from the tail vein of unanesthetized rats just prior to and 30, 45, 75 and 165 minutes after the injection of insulin. The animals were restrained by wrapping them in a towel. To improve bleeding, the base of the tail was infiltrated with 0.5 to 1 ml. of a 2% solution of Intracaine (Squibb). The blood sugar was determined by the Somogyi-Nelson method (Somogyi, 1952).

RESULTS

1. *Effects of somatotrophin (STH) and/or cortisone on the insulin sensitivity of intact rats (Table 1)*

Treatment with the standard dose of STH or cortisone failed to exert any significant influence on the sensitivity of intact rats to 0.1 unit of insulin/kg. of body weight. When 1 unit/kg. of insulin was given, the maximum fall of the blood sugar, which was of the order of 43 mg.% in such animals, was not diminished by pre-treatment of the rats with the standard dosage of STH or cortisone, but an accelerated recovery from the insulin-induced hypoglycemia did occur. Neither STH nor cortisone alone exerted a significant effect on the fasting blood sugar levels. On the other hand, when a combination of the standard dose of STH and cortisone was given, a significant elevation of the fasting blood sugar level was observed and a greater fall of the blood sugar in response to the admin-

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[4] Somatotrophin (Armour lots #R-285-12 and 285183) was generously supplied by Dr. S. L. Steelman of the Armour Laboratories, Chicago, Illinois.

[5] Cortisone (Cortone acetate, Merck) was kindly contributed by Dr. C. A. Winter of the Merck Institute for Therapeutic Research, Rahway, New Jersey.

[6] Iletin (Insulin, Lilly). This preparation contains the hyperglycemic factor.

TABLE 1. EFFECTS OF STH AND/OR CORTISONE ON THE INSULIN SENSITIVITY OF INTACT RATS

Group	Insulin (unit/kg.)	No. rats	Initial blood glucose (mg. %)	Time after insulin—minutes				Maximum drop*
				30	45	75	165	
				Change in blood glucose—mg. %				
Intact	0.1	23	72±2†	+1±2	−8±2	−13±2	−8±2	18%
Intact+STH	0.1	9	71±2	+3±2	−4±3	−11±3	−9±2	16%
Intact+cortisone	0.1	7	79±3	−1±2	−6±1	−8±2	−5±1	10%
Intact	1.0	5	74±1	−20±2	−33±2	−43±3	−37±2	58%
Intact+STH	1.0	6	69±1	−18±4	−34±5	−43±2	−20±2	62%
Intact+cortisone	1.0	12	65±2	−17±2	−27±2	−39±2	−20±3	60%
Intact+cortisone+STH	1.0	8	96±3	−40±3	−49±3	−63±3	−39±3	66%

* Expressed as % of initial blood sugar value.
† Mean ± standard error.
In this and the following tables the regimens are indicated as follows:
STH = 0.5 mg. of Armour standard equivalent/100 gm./day for 5 days
STH/10 = 0.05 mg. of Armour standard dquivalent/100 gm./day for 5 days
cortisone = 0.5 mg./100 gm./day for 5 days
cortisone/10 = 0.05 mg./100 gm./day for 5 days

istration of 1 unit/kg. of insulin ensued. This apparent increase in insulin sensitivity was much less pronounced when the fall in blood sugar was expressed as % of the initial level. It can be concluded that under the conditions of these experiments STH and/or cortisone failed to impart insulin resistance to normal rats.

2. *Effect of hypophysectomy and treatment with STH and/or cortisone on insulin sensitivity*

Hypophysectomized rats showed the expected lowering of the fasting blood sugar level and greatly exaggerated sensitivity to insulin (Table 2). In fact, 9 of the 15 rats in this group developed hypoglycemic convulsions between 75 and 165 minutes after the injection of insulin and 5 of these succumbed before blood could be withdrawn for the final sugar determination. The standard dose of STH, while having no effect on the fasting blood sugar level, markedly diminished the insulin sensitivity of hypophysectomized rats (without, however, restoring it to normal) and prevented the occurrence of physical signs of hypoglycemia.

One-tenth of the standard dose of cortisone elevated (but did not restore to normal) the fasting blood sugar level and the insulin tolerance, thereby

TABLE 2. EFFECT OF HYPOPHYSECTOMY AND TREATMENT WITH STH AND/OR CORTISONE ON INSULIN SENSITIVITY

Group	No. rats	Initial blood glucose (mg. %)	Time after insulin—minutes				Maximum drop*
			30	45	75	165	
			Change in blood glucose—mg. %				
Intact	23	72±2†	+1±2	+8±2	−13±2	−8±2	18%
Hypex.	15	52±2	−12±2	−28±4	−36±3	−37±3‡	71%
Hypex.+STH	10	51±2	+1±2	−10±3	−21±2	−15±3	41%
Hypex.+1/10 cortisone	22	61±2	−10±1	−20±1	−26±1	−22±2	43%
Hypex.+cortisone	14	84±3	−21±2	−34±3	−43±2	−38±2	51%
Hypex.+STH+cortisone	8	73±2	−4±2	−10±3	−10±3	−4±2	14%

* Expressed as % of initial blood sugar value.
† Mean ± standard error.
‡ 10 rats.
For details of regimens see Table 1.

TABLE 3. EFFECTS OF HYPOPHYSECTOMY AND TREATMENT WITH VARIOUS REGIMENS OF STH AND/OR CORTISONE ON INSULIN SENSITIVITY

Group	No. rats	Initial blood glucose (mg. %)	Time after insulin—minutes				Maximum drop*
			30	45	75	165	
			Change in blood glucose—mg. %				
Hypex.	15	52±2†	−12−2	−28±3	−36±3	−37±3	71%
Hypex.+1/10 STH	7	62±3	−14−2	−19±2	−31±3	−28±3	50%
Hypex.+1/10 cortisone	22	61±2	−10−1	−20±1	−26±1	−22±2	43%
Hypex.+1/10 STH+1/10 cortisone	12	63±3	−4−2	−16±2	−24±2	−16±2	38%
Hypex.+1/10 STH+cortisone	11	95±2	+8−2	−13±1	−19±2	−6±2	20%
Hypex.+STH	10	51±1	+1+2	−10±3	−21±2	−15±3	41%
Hypex.+STH+1/10 cortisone	11	64±2	+3±3	−5±2	−13±3	−8±2	20%
Hypex.+STH+cortisone	8	73±2	−4±2	−10±3	−10±3	−4±2	14%

* Expressed as % of initial blood sugar value.
† Mean ± standard error.
For details of regimens see Table 1.

preventing hypoglycemic convulsions. When the standard dose of cortisone was given, fasting blood sugar levels rose to higher than normal values, but insulin sensitivity was not further decreased. On the contrary, the absolute blood sugar fall was markedly greater than in hypophysectomized rats on the low cortisone regimen. However, because of the high starting blood sugar level severe hypoglycemia did not develop.

When hypophysectomized rats received the standard dosage of STH in addition to the standard cortisone regimen, both the elevated fasting blood sugar level and the enhanced insulin sensitivity returned to normal. This indicated that, in hypophysectomized rats, STH and cortisone can be antagonistic as regards glycemia and at the same time be markedly synergistic as insulin-antagonists. Further experiments in which the dosage of STH and cortisone was varied (Table 3) were in general conformity with this finding, although they indicated that 0.1 the standard dose of STH did not always modify the effects of cortisone substantially.

3. *Effect of adrenalectomy and treatment with STH and/or cortisone on insulin sensitivity (Table 4)*

Adrenalectomized rats displayed fasting hypoglycemia and enhanced

TABLE 4. EFFECT OF ADRENALECTOMY AND TREATMENT WITH STH AND/OR CORTISONE ON INSULIN SENSITIVITY

Group	No. rats	Initial blood glucose (mg. %)	Time after insulin—minutes				Maximum drop*
			30	45	75	165	
			Change in blood glucose—mg. %				
Intact	23	72±2†	+1±2	−8±2	−13±2	−8±2	18%
Adx.	13	57±2	−6±1	−20±2	−33±2	−37±2‡	65%
Adx.+STH	12	63±4	−10±2	−20±2	−32±2	−30±4§	51%
Adx.+cortisone	9	73±2	−2±2	−8±2	−10±2	0±1	14%
Adx.+1/10 cortisone	10	65±1	−6±3	−19±2	−24±2	−13±3	38%
Adx.+1/10 cortisone+STH	8	69±1	−11±4	−17±5	−28±5	−16±6	41%

* Expressed as % of initial blood sugar value.
† Mean ± standard error.
‡ 4 rats.
§ 9 rats.
For details of regimens see Table 1.

insulin sensitivity. Hypoglycemia signs occurred at higher blood sugar levels than in hypophysectomized animals, and only 4 of 13 rats survived the experimental period.

Treatment with the standard dose of STH failed to have any statistically significant effect on the fasting blood sugar concentration or the insulin sensitivity of adrenalectomized rats. However, since the initial blood sugar was somewhat higher and the maximum blood sugar fall slightly less in the STH-treated rats, the hypoglycemic nadir was significantly higher in this group than in the untreated adrenalectomized animals. This may have been at least in part responsible for the fact that STH alleviated the proneness of adrenalectomized rats to hypoglycemic convulsions, inasmuch as only 3 of the 12 rats thus treated succumbed before the last blood sample could be withdrawn.

The standard dose of cortisone elevated the fasting blood sugar concentration of adrenalectomized rats without, however, raising it to abnormal levels, as it did in hypophysectomized rats. It also abolished the exaggerated sensitivity of these animals to insulin. In fact, the recovery of the blood sugar levels after the administration of the test dose of insulin appeared to be faster in this group than in normal rats.

In order to examine whether an STH-cortisone synergism with respect to anti-insulin action (similar to that observed in hypophysectomized rats) could be seen in adrenalectomized rats, the following experiment was carried out. One group of adrenalectomized rats was injected with 0.1 of the standard dose of cortisone, which did not completely restore their fasting blood sugar and insulin sensitivity to normal. Another group of animals received this dose of cortisone together with the standard dosage of STH. It is evident from Table 4 that this combination of the two hormones was no more effective in inhibiting the exaggerated hypoglycemic response to insulin than was cortisone alone.

4. *Effect of STH on the insulin sensitivity of hypophysectomized-adrenalectomized rats* (*Table 5*)

Hypophysectomized-adrenalectomized rats, as well as their hypophysectomized-sham adrenalectomized controls, showed a lower fasting blood sugar level than hypophysectomized rats, and a hypoglycemic response to insulin which was remarkably sluggish as compared with that of hypophysectomized or adrenalectomized animals. Nevertheless, by the 165 minute interval one of the 11 hypophysectomized-adrenalectomized rats and 3 of the 8 hypophysectomized-sham adrenalectomized rats had succumbed, and the survivors showed severe hypoglycemia. None of the 15 hypophysectomized-adrenalectomized rats that were treated with the standard dose of STH developed fatal hypoglycemia or even hypoglycemic signs, and their blood sugar did not diminish after the 75 minute

TABLE 5. EFFECT OF STH ON THE INSULIN SENSITIVITY OF HYPOPHYSECTOMIZED-ADRENALECTOMIZED RATS

Group	No. rats	Initial blood glucose (mg. %)	Time after insulin—minutes				Maximum drop*
			30	45	75	165	
			Change in blood glucose—mg. %				
Hypex.-sham adx.	8	44 ± 4†	−6 ± 3	−10 ± 4	−18 ± 4	−32 ± 6‡	73%
Hypex.-adx.	11	43 ± 2	−2 ± 1	−5 ± 1	−13 ± 2	−24 ± 2§	55%
Hypex.-adx. + STH	15	42 ± 2	−3 ± 1	−8 ± 2	−13 ± 2	−11 ± 1	31%

* Expressed as % of init al blood sugar value.
† Mean ± standard error.
‡ 5 rats.
§ 10 rats.
For details of regimen see Table 1.

interval as it did in the untreated rats. Somatotrophin therefore protected hypophysectomized rats against the effects of insulin on the blood sugar even when the adrenals were not present.

DISCUSSION

The criteria of insulin sensitivity

It is problematic which of the following criteria should be considered the most important in evaluating the response of the blood sugar to a test dose of insulin: 1) the rate of decline of the blood sugar level; 2) the magnitude of the maximal change in this level expressed a) in absolute terms or b) as % of the initial blood sugar value; 3) the level of the hypoglycemic nadir; or 4) the rate of restitution of normoglycemia. In the present paper the tables permit the study of all these parameters of the change in the blood sugar induced by insulin. However, the true slope of the blood sugar fall is obscured by the presence of the hyperglycemic factor in the insulin preparation used. Whereas in most conditions the maximal hypoglycemic response expressed in absolute terms appears to be a convenient and useful measure of insulin sensitivity, it may not be a reliable index when initial blood sugar levels are elevated (e.g., in normal rats treated with somatotrophin (STH) and cortisone, or in hypophysectomized rats receiving higher doses of cortisone). In these conditions the absolute change in glycemia may suggest markedly increased insulin sensitivity, albeit less profound or no significant deviations from the normal pattern are found if the blood sugar fall is expressed as % of the initial glucose level or if the blood sugar nadir is examined.

Comparison of our observations on glycemia and insulin sensitivity with those of previous workers

Although insulin resistance was produced by large doses of STH in normal rats (Milman and Russell, 1950), the substantially lower "standard" dosage of the hormone used in this study failed to have such an effect.

However, the same dose of STH was beneficial in ameliorating the insulin sensitivity of hypophysectomized rats. This effect of STH after hypophysectomy is in agreement with the results obtained by de Bodo and Sinkoff (1953a) in dogs. Restoration of the normal insulin tolerance was not achieved with the standard STH regimen in hypophysectomized rats. Perhaps higher doses of the hormone would have further alleviated the insulin sensitivity due to ablation of the pituitary. On the other hand, the standard dose of STH did not influence the hypoglycemia of fasting in hypophysectomized rats, and larger doses are even known to produce hypoglycemia in intact rats (Milman and Russell, 1950). It is therefore unlikely that normal glycemia and insulin tolerance could both be obtained by the treatment of hypophysectomized rats with STH alone. De Bodo and co-workers were also unable to normalize the carbohydrate metabolism of hypophysectomized dogs by administering only STH. In that species, however, the difficulty was caused by the tendency of the animals thus treated to develop a) diabetic glucose tolerance patterns, b) toxic signs or c) resistance to the effects of the hormone (de Bodo and Sinkoff, 1953a, de Bodo and Altszuler, 1955).

Cortisone (0.1 of the standard dose) also abated the insulin sensitivity of hypophysectomized rats. This is in harmony with de Bodo and Sinkoff's (1953a) findings in dogs, as is the observation that replacement therapy with cortisone alone was not feasible in hypophysectomized animals. Administration of the standard dose of cortisone resulted in an apparent or real augmentation rather than further alleviation of insulin sensitivity, whereas the blood sugar level became higher than normal. In intact rats the same dose of cortisone did not produce hyperglycemia.

In our hypophysectomized rats, as in the hypophysectomized dogs of de Bodo and Sinkoff (1953a), the combination of STH with cortisone was required to restore to normal those parameters of the disturbed carbohydrate metabolism which were investigated. However, in de Bodo's dogs cortisone checked the diabetogenic and toxic effects of STH, whereas in hypophysectomized rats the hyperglycemic effect of cortisone was counteracted by appropriate doses of STH. With regard to insulin sensitivity STH and cortisone were markedly synergistic in hypophysectomized rats. De Bodo and Sinkoff (1953a) stated that STH tended to render hypophysectomized dogs insulin resistant, and that normal insulin sensitivity was achieved by concurrently given cortisone. However, in a more recent review de Bodo and Altszuler (1955) emphasized that the addition of STH enhanced the anti-insulin effect of cortisone in hypophysectomized dogs. It is therefore questionable whether a fundamental difference exists between rats and dogs as regards the synergistic anti-insulin action of STH and cortisone in the absence of the pituitary.

A synergism between STH and corticoids has also been observed by several workers who studied other facets of carbohydrate metabolism.

Notably, a) adrenal cortical extract potentiated the inhibitory action of STH on glucose uptake by isolated rat diaphragms, provided that both substances were injected *in vivo* (Park *et al.*, 1952); b) combined STH-corticoid regimens were necessary to decrease the accelerating action of tissue-bound insulin on glucose uptake *in vitro* if the donors were either hypophysectomized or adrenalectomized (Stadie, 1951); c) glycogen deposition from fed glucose was promoted in adrenalectomized rats by STH only if corticoids were also administered (Illingworth and Russell, 1951); d) ACTH enhanced the diabetogenic action of STH in cats (Reid, 1953); e) a lipoprotein inhibitor of glucose uptake by diaphragms *in vitro* was found in the serum of diabetic rats, but in that of diabetic-hypophysectomized animals only if they were injected with both STH and corticoids (Bornstein and Park, 1953); and f) subdiabetogenic doses of ACTH rendered force-fed rats diabetic when given together with STH which by itself did not produce diabetes (Engel *et al.*, 1952). The latter finding is consistent with our observation that the combination of STH and cortisone regimens, neither of which alone affected glycemia, raised the fasting blood sugar in normal rats. On the other hand, in the hypophysectomized animal, in which cortisone alone led to hyperglycemia, this effect was counteracted rather than abetted by STH. It is of interest that Owen *et al.* (1955) recently reported that the hyperglycemia produced by *diabetogenic* doses of cortisone in intact rats was diminished by STH, whereas STH treatment evoked diabetes in synergism with a *subdiabetogenic* cortisone regimen. They suggested that these variable effects of the combination of the two hormones may be due to the fact that STH can a) antagonize the influence of cortisone on the blood sugar level by inhibiting gluconeogenesis, which this steroid promotes, or b) enhance the hyperglycemic effect of cortisone by synergistic suppression of glucose utilization in tissues. It is not clear why, under the conditions of our experiments, the former mechanism appeared to be operative in hypophysectomized rats and the latter in the intact animals.

In adrenalectomized rats the standard dose of cortisone apparently represented adequate replacement therapy, as judged by its effects on glycemia and insulin tolerance. One-tenth of the standard dose was less effective, but nevertheless obviously beneficial. An effect of STH on the hypersensitivity of adrenalectomized rats to insulin could not be demonstrated with the dosage used in this study. This is in contrast with the findings of Lazarus and Volk (1952) in the dog. Higher doses of STH may temper the insulin sensitivity of adrenalectomized rats, but it is dubious whether such an effect can be convincingly demonstrated, as the hypoglycemia elicited by such a regimen tends to be pernicious in the absence of the adrenals (Milman and Russell, 1950). Since some of the effects of STH on carbohydrate metabolism are known to be dependent on at least traces of corticoids (*vide supra*), the possibility had to be considered that

the adrenalectomized rat is refractory to the anti-insulin action of STH because of its lack of cortical steroids. However, STH did not modify the blood sugar response in adrenalectomized rats receiving incomplete replacement therapy with cortisone. Furthermore, the idea that corticoids may be indispensable for the anti-insulin effect exerted by STH was contradicted by the finding that STH protected hypophysectomized-adrenalectomized rats against the hypoglycemic action of insulin. Similar observations were made on hypophysectomized-adrenalectomized dogs by Sinkoff *et al.* (1954).

Possible effects of electrolyte balance and nutritional status on the response to insulin

The fact that hypoglycemic convulsions occurred at higher blood sugar levels in adrenalectomized rats than in hypophysectomized rats may have been due to the lack of mineralocorticoids in the former. Woodbury (1954) has shown that steroids which are potent preservers of body sodium decrease the excitability of the brain in rats. The apparent beneficial influence of STH in preventing the physical signs of insulin hypoglycemia in adrenalectomized rats may also have been partly due to the sodium retention which this hormone brings about (Stein *et al.*, 1952).

In hypophysectomized-adrenalectomized rats the blood sugar fall after the injection of insulin was remarkably sluggish. This could not have been due to the removal of the adrenal *per se*, since hypophysectomized-sham adrenalectomized controls exhibited the same type of response. It must be pointed out that the fasting blood sugar was lower in these rats than in hypophysectomized animals. Apparently both adrenalectomy and sham adrenalectomy, when performed in hypophysectomized rats, decreased the food intake; this led to hypoglycemia, which in turn seems to have resulted in a metabolic adaptation reflected by the slow drop of the blood sugar level after insulin administration. Such findings again emphasize the importance of the nutritional status of the experimental animals in endocrine-metabolic studies (Samuels, 1947, Ingle, 1951, Soskin and Levine, 1952). The fact that food intake was not standardized in the rats of this study (except for the final 24 hour fast) must be considered a shortcoming of the experimental design. However, even controlled food ingestion does not eliminate variations in food utilization due to the vagaries of absorption from the intestine.[7]

Interpretation of hormonal effects on insulin sensitivity

Because of the complex nature of blood glucose homeostasis, any at-

[7] Recent studies on the insulin sensitivity of underfed intact rats (Spirtos and Halmi, 1955) showed a pattern different from that seen in any of the present experimental groups, viz., a higher than normal fasting blood sugar level and increased sensitivity to insulin which was remedied by cortisone but not by STH.

tempt at an interpretation of insulin sensitivity must be regarded as tentative. There is little doubt that insulin causes a fall in blood sugar primarily by enhancing the rate of withdrawal of glucose from the blood, and apparently also by diminishing hepatic glucogenesis (Bearn, Billing and Sherlock, 1953). Recovery from the hypoglycemia induced by a test dose of insulin appears to be due mainly or exclusively to an activation of hepatic glycogenolysis in direct response to the lowering of the blood sugar level (Soskin and Levine, 1952). The effects exerted by hormones on the sensitivity to insulin may be due a) to modification of the influence of insulin in enhancing peripheral glucose utilization; b) to changes in the concentration and/or availability for glucogenesis of hepatic glycogen stores (Lazarus and Volk, 1953).

The greatly decreased insulin tolerance of hypophysectomized rats is apparently due a) to an approximately fourfold increase in the response of the peripheral glucose uptake to insulin (Bennett and Roberts, 1946) and b) to depletion of the hepatic glycogen stores during fasting (Long *et al.*, 1940). It is understandable that the hypophysectomized rats given 0.1 of the standard dose of cortisone showed a definite improvement of their insulin sensitivity, since glucocorticoids are known to increase the concentration of liver glycogen whether the pituitary is removed or not (Long *et al.*, 1940). Hypophysectomized rats treated with the standard dose of cortisone had higher than normal blood sugar levels after a 24 hour fast and, presumably, liver glycogen stores at least as high as those of normal fasted rats. Nevertheless, their blood sugar fall in response to 0.1 unit of insulin per kg. was comparable with the reaction of intact animals to 1 unit per kg., i.e., their insulin sensitivity was approximately ten times normal. It is not clear whether the increased response of peripheral glucose utilization to insulin can fully account for the diminished insulin tolerance seen in these cortisone treated hypophysectomized rats, or whether it is necessary to assume that the "hypoglycemia responsiveness" of the hepatic glycogenolytic mechanism is also defective in the absence of the pituitary, even though corticoids be administered. Glycogenolysis after the injection of adrenaline is normal in the liver of hypophysectomized rats (Russell and Cori, 1937). The effect of STH on the insulin sensitivity of hypophysectomized rats can be explained on the basis of studies on the isolated diaphragm (Krahl, 1951). The insulin-induced increase in the uptake of glucose by this muscle was smaller if the tissue was obtained from a hypophysectomized-STH treated rat than if the donors were untreated. The possibility that STH also influences the "hypoglycemia responsiveness" of the liver remains to be explored. The liver glycogen level of fasted hypophysectomized rats is not altered by STH (Illingworth and Russell, 1951).

The insulin hypersensitivity of fasted adrenalectomized rats, which is completely abolished by an appropriate cortisone regimen, appears to be

due mainly to the diminished hepatic glycogen stores which characterize this condition (Long *et al.*, 1940). We are not aware of any work done on the insulin tolerance of adrenalectomized-eviscerated rats, but Villee and Hastings (1949) found that insulin is more effective in promoting the uptake of labelled glucose by the diphragm of adrenalectomized rats than it is in the case of intact donors. A subsidiary role of glucose overutilization in the decreased insulin tolerance of adrenalectomized animals cannot therefore be categorically denied. However, there does not appear to be a disturbance in carbohydrate metabolism after adrenalectomy that is specifically remedied by STH, as there is after hypophysectomy. The fact that large doses of STH may have had some effect on the insulin sensitivity of adrenalectomized rats (Milman and Russell, 1950) is not in contradiction with this statement, since such a regimen can make normal rats insulin resistant. The marked amelioration of insulin tolerance in adrenalectomized dogs treated with STH (Lazarus and Volk, 1952) also appears to have been a manifestation of insulin resistance rather than a therapeutic effect on the specific metabolic defect.

The observed anti-insulin synergism between STH and cortisone in hypophysectomized rats is easily explained, even though we may not understand all its facets. If STH curbs exaggerated glucose utilization and cortisone elevates hepatic glycogen stores, the result must be a more temperate hypoglycemic response to insulin than is obtained when either of these hormones is given alone. This hormonal synergism did not occur, under the conditions of our experiments, in normal or adrenalectomized rats, in which an enhancement of glucose utilization that responds specifically to STH therapy is not encountered.

In conclusion, the present studies on the insulin sensitivity of rats indicate that in this species, as in the dog (de Bodo and Sinkoff, 1953a), STH plays a physiologic role distinct from that of corticoids in the regulation of carbohydrate metabolism.

SUMMARY

The hypoglycemic response to insulin (0.1 to 1.0 unit/kg. body weight administered intraperitoneally after 24 hours of fasting) was determined in rats subjected to various experimental procedures.

Our "standard" regimens of somatotrophin (STH) and cortisone (STH: 0.5 mg. Armour standard equivalent/100 gm. body weight daily for 5 days; cortisone: 0.5 mg./100 gm. body weight daily for 5 days) were not individually effective on either the fasting blood sugar or the insulin sensitivity of intact rats, whereas fasting hyperglycemia and a greater absolute drop of the blood sugar in response to insulin occurred when a combined STH-cortisone regimen was given.

Hypophysectomized rats showed low fasting blood sugar levels and a greatly exaggerated sensitivity to insulin. The standard dose of STH and

smaller quantities of cortisone (0.1 of the standard dose), while having no or little effect on the fasting hypoglycemia, partially counteracted the insulin sensitivity of such animals. When the standard regimen of cortisone was given, the insulin sensitivity of hypophysectomized rats increased as the fasting blood sugar became higher than normal. Somatotrophin and cortisone exerted opposite effects on the fasting blood sugar level of hypophysectomized rats (in contrast to their action in intact animals). However, there was a marked synergism between these hormones with regard to insulin-antagonism in hypophysectomized rats.

Adrenalectomized rats also displayed fasting hypoglycemia and enhanced insulin sensitivity. Cortisone (standard dose) restored the fasting blood sugar and the insulin sensitivity of these animals to normal, whereas the standard STH regimen had no effect on either. STH was also ineffective when given in conjunction with incomplete replacement therapy with cortisone (0.1 of the standard dose).

Hypophysectomized-adrenalectomized rats, as well as their hypophysectomized-sham adrenalectomized controls, showed lower fasting blood sugar levels than hypophysectomized rats, and a remarkably sluggish—but nevertheless greatly exaggerated—blood sugar response to insulin.

STH (standard dose) protected hypophysectomized-adrenalectomized rats against the hypoglycemic effects of insulin, without affecting the fasting hypoglycemia.

It is concluded that the metabolic defect which leads to insulin sensitivity is not the same in hypophysectomy and in adrenalectomy, since the insulin sensitivity of hypophysectomized animals is synergistically alleviated by STH and cortisone, whereas that of adrenalectomized animals responds only to cortisone therapy. The findings support the thesis that STH plays an important role in the carbohydrate metabolism of the normal animal.

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TOTAL GONADOTROPHIC POTENCY OF MULE DEER PITUITARIES[1]

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A COOPERATIVE study of reproductive phenomena in mule deer at the National Bison Range, Moiese, Montana was initiated in the fall, 1953 by the Department of Zoology, Montana State University and the Montana Wildlife Research Unit cooperating with the U. S. Fish and Wildlife Service and the Montana Fish and Game Department.[2]

A deer herd reduction program was to be instituted on the National Bison Range for management purposes. In order to take advantage of this material prompt action was necessary. It was agreed that part of the reduction program could be spread over a period of one year in order that autopsy material could be collected every month of the year by us.

The principal objective of the present investigation is to report on the total gonadotrophic potency of entire pituitaries of female mule deer (*Odocoileus hemionus hemionus*) collected from three different age groups throughout the year. Reproductive tracts, endocrine glands, and other tissues were also collected. The analysis of material in these latter categories will be reported elsewhere.

MATERIAL AND METHODS

Female mule deer (fawns, 1 week to 11 months of age; yearlings, 12 to 23 months; and adults, 24 months or older) were shot on the National Bison Range according to a pre-arranged schedule. Attempts were made to collect one of each of the above age groups each month of the year, with collections weekly or biweekly during the months of October, November, and December.

The entire pituitary gland was dissected from the fresh carcass in the field, trimmed down to the connective tissue capsule, and placed in a vial containing an excess of fresh acetone. All pituitaries were removed less than three hours after death—usually within an hour and a half after death. Fresh weights of 14 pituitaries were taken in the field before being placed in the acetone. Upon arrival at the laboratory all pituitaries were placed in fresh acetone and stored in a refrigerator.

A total of 61 pituitaries was collected from 20 fawns, 19 yearlings, and 22 adults.

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[2] We wish to acknowledge with gratitude the splendid cooperation of the following agencies without whose assistance this study could not have been carried out: U. S. Fish and Wildlife Service, Montana Cooperative Wildlife Research Unit, and the Montana Fish and Game Department.

After the acetone dry weight of the whole pituitary, with the connective tissue capsule removed, was taken the whole gland was pulverized with mortar and pestle. It was found that 1 mg. of dry pituitary powder was equivalent on the average to 6.58 mg. of fresh pituitary (Table 1).

Whole pituitary powder suspensions were made using 2.5 mg. of powder per cc. of sterile physiological saline. These suspensions were prepared immediately before the first injection into the bioassay animals and were kept under refrigeration between injections. A total dose of 10 mg. of pituitary powder was administered in twice daily doses over a period of four days into 20–21-day-old intact female mice. Intact immature

TABLE 1. MONTH OF COLLECTION, FIELD DRESSED WEIGHTS, AND PITUITARY GLAND WEIGHTS OF FEMALE MULE DEER COLLECTED ON THE NATIONAL BISON RANGE, MOIESE, MONTANA

Age group	Month of collection	Number of deer	Avg. body weight	Avg. age	Avg. pituitary weights	
					Fresh field	Acetone dry
			(lbs.)	(mo.)	mg.	mg.
Fawn	June–Aug.	3	17.7	1.5	281.0 (1)*	33.3
	Sept.–Nov.	7	39.9	4.5	254.0 (1)	29.6
	Dec.–Feb.	7	30.4†	6	—	28.4
	Mar.–May	3	46.3	9.5	351.0 (1)	41.8
Yearling	June–Aug.	3	61.5	13	452.5 (3)	72.7
	Sept.–Nov.	7	75.0	16	318.0 (1)	54.3
	Dec.–Feb.	8	72.4†	19	—	54.8
	Mar.–May	2	72.0	23	506.2 (1)	94.0
				(yrs.)		
Adult	June–Aug.	3	89.0	2.5	741.6 (3)	128.9
	Sept.–Nov.	9	91.9	3.5	535.0 (1)	76.6
	Dec.–Feb.	7	80.4†	4	—	91.9
	Mar.–May	4	82.8	6	1155.0 (1)	126.9

* Number in ( ) refers to number of actual fresh field weights.
† Note low body weights of Dec.–Feb. kills.

female mice are far less desirable for assay of pituitary substance than are hypophysectomized animals, but circumstances were such that only immature normal mice were available for purposes of this study. However every effort was made to have normal litter mate controls for every bioassay series.[3]

The 20–21-day-old controls were injected with an equivalent amount of sterile physiological saline. The bioassay mice and controls were autopsied on the fifth day after the initial injection. Control and experimental mice were always injected and assayed simultaneously to reduce variations from possible effects in the ration of meat meal prepared from steers fattened with stilbestrol supplement.

The response of the uterine and ovarian weights of the bioassay animals was expressed as a per cent of increase of the experimentals over the saline injected controls. This figure was arrived at by first reducing the average uterine weight to a per cent of the mean body weight for each bioassay series and using these figures in the following formula:

$$\frac{\text{Exp. wt.} - \text{control wt.}}{\text{control wt.}} \times 100 = \%\ \text{increase.}$$

Ovarian weights of bioassay mice remained essentially unchanged.

[3] The original stock of inbred mice was kindly provided us by the Microbiological Institute, U.S.P.H.S., Hamilton, Montana.

For purposes of standardization, graduated doses of a commercial pregnant mare serum were assayed on a series of mice. Similarly, a purified FSH preparation from sheep anterior pituitary was also assayed, using the same criteria as for the deer pituitaries, namely increased uterine weight of experimental mice over the uterine weight of control mice (Table 2).

The responses elicited from the use of the entire deer pituitaries are not interpreted to indicate the amount of FSH or LH in the deer pituitaries but to indicate a total gonadotrophic response. The same criteria for potency was used for the FSH preparation, the PMS solution, and the powdered deer pituitaries.

TABLE 2. BIOASSAY OF PMS AND FSH

PMS[4]				FSH[4]			
Total dose	No. of mice		% increase uterine wt.	Total dose	No. of mice		% increase uterine wt.
	Exp.	Control			Exp.	Control	
0.10 mg.	5	6	0	0.5 mg.	6	6	10.1
0.50 mg.	3	8	22.9	1.0 mg.	7	6	56.1
0.75 mg.	5	5	82.7	2.0 mg.	7	7	218.9
1.00 mg.	6	9	420.9	4.0 mg.	6	6	491.6

RESULTS

Bioassays of the fawn and yearling pituitaries show essentially the same pattern throughout the year and will be considered together. Injections of fawn and yearling female deer pituitary substance into intact immature female mice yielded the greatest uterine response when the pituitaries from the August–September collections were used (Fig. 1). The maximum numbers of follicles, 2 mm. or more in diameter, were found in the donor deer fawns and yearlings collected during August and September (Fig. 1).

The highest equivalent uterine response calculated for fawn and yearling pituitaries in terms of the FSH and LH standards took place in the August–September collections and was equivalent to 1.85 mg. of FSH powder, and .82 mg. of LH powder. For the adult does the highest equivalent uterine response calculated was from the November–December kills and was equivalent to 1.50 mg. of FSH and .82 mg. of LH preparations.

The minimum uterine response occurred in the female mice injected with pituitary substance from the fawns and yearlings killed during January and February. The January–February fawns and yearlings also showed the smallest numbers of ovarian follicles, 2 mm. or more in diameter (Fig. 1). The complete ovarian analysis from which the follicle count was obtained is reported elsewhere (Sears, 1955).

The decline in pituitary potency of the yearling doe, as indicated by the mouse uterine reaction, is more gradual than is the decline in potency of

[4] The PMS was commercial Anteron, Schering's gonadotrophic hormone. The FSH was a purified preparation of sheep anterior pituitary (FSH S67A 2/13/53; 7.22 mg. is 1 gm. equivalent) furnished through the courtesy of W. H. McShan, Dept. of Zoology, Univ. of Wisconsin.

the fawn pituitary (Fig. 1). The yearlings have a higher pituitary potency in December than do the fawns.

The pattern of pituitary potency of the adult doe is somewhat different from that exhibited by the fawns and yearlings. Immature female mice yielded maximum uterine response in the bioassay when the pituitaries were obtained from adult does autopsied in late November and early December. Pituitaries collected at this time had higher potencies than the

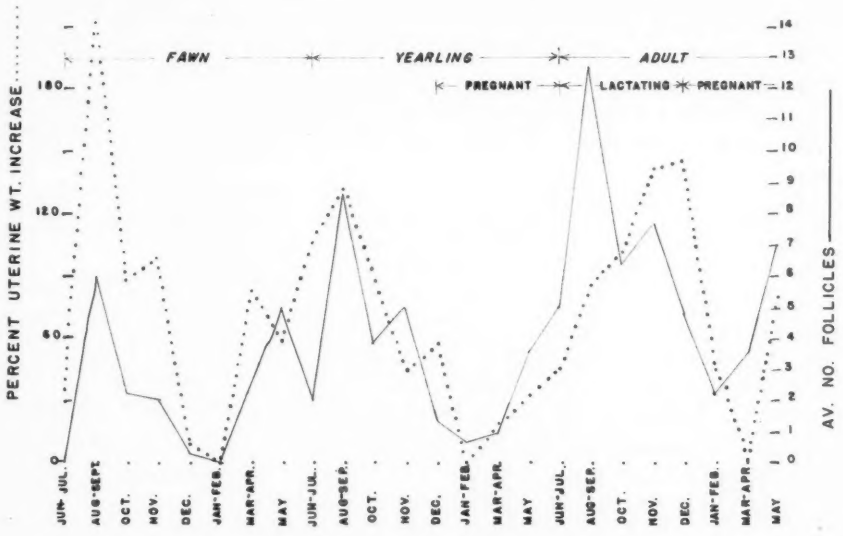

FIG. 1. The average pituitary gonadotrophic potency, and the average number of ovarian follicles, 2 mm. or larger, for three age classes of mule deer

Per cent uterine wt. increase · · · · · · · · · · .

Av. No. Follicles————————————

pituitaries collected from deer killed in late October, early November and the last half of December.

Interestingly enough the count of follicles, 2 mm. or more, in the adult deer ovaries was like that of the fawns and yearlings, namely that the highest count was in the ovaries of adult does collected during August and September (Fig. 1). The lowest count of follicles, 2 mm. or over, was in the adult doe ovaries recovered from the January-February kills.

The minimum in the curve of annual pituitary potency was later (March–April) in the adult doe pituitaries than in the curve of potency for the fawn and yearlings (January–February, Fig. 1).

DISCUSSION

Briefly reviewing the reproductive cycle of the female mule deer it is safe to say that mule deer fawns rarely breed. There are however four

cases reported in the literature which indicate that mule deer fawns do occasionally become pregnant. The yearlings breed at 17–19 months of age. Thirty-one out of thirty-six yearlings autopsied between December 12th and May 14th were pregnant (Sears, 1955). Thirty-eight out of thirty-nine adult does autopsied during this same period were also found to be pregnant.

Pregnancy may begin in the mule deer by mid-November (the first corpus luteum of ovulation was found November 14th) but more frequently during late November and continues until late May or early June. Lactation continues until late November or early December when the doe again becomes pregnant (the earliest macroscopic 4 mm. embryo was found in a December 8th kill; Paul Hudson, personal communication). Milk can no longer be expressed from the nipples of the adult doe in late December.

The peak counts of follicles, 2 mm. or over, was found to take place in August in all three age groups, and the lowest counts in all three classes took place in January and in February.

Examination of Figure 1 shows a good agreement throughout the year ($r = .57$) in all but the adult does between the total gonadotrophic potency of the pituitaries of female mule deer and the average number of follicles over 2 mm. in diameter. Robinson and Nalbandov (1951) state that the number of follicles in the sow parallels closely the rise or fall in pituitary potency. Nalbandov and Casida (1940) report that there is an average decline in follicular development in pregnant cows paralleling the decline in gonadotrophic activity of the pituitary.

Kammlade, Welch, Nalbandov, and Norton (1952) report that in the ewe there were significantly more follicles during the breeding season when pituitary potency was comparatively low than there were in the ovaries recovered from ewes during anoestrous when the pituitary potency was reported to be relatively high. They found no correlation between the number of follicles and the stage of the estrous cycle in the ewe. In the adult mule deer we found that the follicle count is high in August while the pituitary potency is highest in late November and early December (Fig. 1).

The peaks of total pituitary gonadotrophic potency in both the fawn and yearling occurred in the August–September collections (Fig. 1). Bates, Riddle, and Lahr (1935) similarly report that veal calves had as high or higher potency than nonpregnant cows or late pregnancy cows.

The maximum gonadotrophic potency of adult deer did not take place until the late November and early December collections. Bates, Riddle, and Lahr (1935), Nalbandov and Casida (1940) report that the maximum gonadotrophic potency during pregnancy in cows is during the early stages of pregnancy. Our data suggest that this may be the case in mule deer. All the adult deer collected from June through October and early

November were lactating and nonpregnant. Adult deer collected in late November had ovulated and were presumably pregnant, and the early December collections were established to be pregnant.

SUMMARY

Whole pituitary glands from 20 fawn does, 19 yearling does, and 22 adult doe deer were bioassayed for gonadotrophic potency using 20–21-day-old intact female mice. The assay results were based on the percentage of uterine weight increase in experimental mice over the uterine weights of normal litter mate control mice.

The results indicate that first summer fawns and yearlings have pituitaries with a high total gonadotrophic potency during the summer months. During the winter months their pituitary potency is low and only gradually increases as spring progresses.

A peak level of gonadotrophic potency is reached in adult (2 years or older) deer by late November and early December. This peak is followed by a sharp decline to a low in February and March slowly rising towards the termination of pregnancy in late May or early June.

Counts of the average number of ovarian follicles, 2 mm. or over, showed a maximum in all three age classes in the August–September kills, and a minimum in all three age classes in the January–February kills.

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THE ANTILUTEINIZING POTENCY OF FIVE DERIVATIVES OF PROGESTERONE

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INTRODUCTION

THE antiluteinizing potency of progesterone known through the work of Dempsey, Selye and others has been tested in experiments with intrasplenic ovarian grafts in castrated female guinea-pigs (Mardones *et al.*, 1951). When estrogen is administered appearance of hemorrhagic follicles in the graft is prevented and large corpora lutea are produced; when progesterone is given simultaneously with estrogen, corpora lutea fail to appear. Evidence has been given that the quantity of progesterone necessary for complete prevention of estrogen-induced corpora lutea is probably smaller than that which is produced by the normal ovary in the luteal phase. Since these results have been obtained in experiments with the *continuous* administration of progesterone (absorbed from pellets) they do not definitely prove that the antiluteinizing faculty of progesterone interferes in the autoregulatory mechanism on which the sequence of the ovarian phases depends; but these results would not be contrary to the concept that the antiluteinizing faculty of progesterone is implicated in such an autoregulatory mechanism. This adds considerable interest to the problem of the comparative antiluteinizing potency of progestational steroids.

In former work the antiluteinizing potency of progesterone (P) has been compared with that of desoxycorticosterone acetate (DCA), ethinyltestosterone (ET) and other progestational compounds. The progestational potency of DCA and ET coincides; it equals about one tenth that of P (Selye and Masson, 1943; Masson and Selye, 1945). However, as to the antiluteinizing potency ET differs greatly from DCA. With quantities of DCA, 4 to 10 times greater than the antiluteinizing quantities of P, an antiluteinizing effect was obtained. On the contrary, with quantities of ET, 3 to 10 and even 20 times those of P, there was no antiluteinizing effect. Results with vinyl-testosterone (VT) and ethinyl-Δ^5-androstenediol were similar to, though not coincident with, those obtained with ET. When considering results with DCA only there is a concomitancy of progestational and antiluteinizing potencies; when considering results with ET and the other mentioned synthetical steroids there is no such concomitancy. The discrepancy between DCA and ET as to their antiluteinizing potency

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offers a most interesting problem which is seemingly fundamental also from a pharmacological point of view: whereas DCA is a steroid normally occurring in the body, ET is structurally outside any normal steroid pattern. Is this deepgoing structural difference decisive as to concomitancy between progestational and antiluteinizing potencies? A study of the comparative antiluteinizing potency of progesterone derivatives is liable to promote understanding in this special field.

EXPERIMENTAL RESULTS

In the present work five different derivatives of progesterone whose progestational and antifibromatogenic potencies have been studied by various authorities have been examined as to their antiluteinizing potency. Two of these steroids, 19-nor-progesterone (19-nor-P) and Δ^{11}-dehydroprogesterone (Δ^{11}-dehydro-P), are more progestational than P (Tullner and Hertz, 1953; Meystre *et al.*, 1948). Both are also antifibromatogenic; the first one, as to this, is much superior to P (Mardones *et al.*, 1954a; Mardones *et al.*, 1953). Two other derivatives, 11-keto-progesterone and 11β-hydroxy-progesterone (11β-OH-P) have only a very slight progestational and antifibromatogenic potency (Byrnes *et al.*, 1954, 1955; Mardones *et al.*, 1954b). With the fifth derivative, 11α-hydroxy-progesterone (11α-OH-P) so far no progestational potency has been established though there was a definite antiestrogenic (antihysterotrophic) action in the rat (Byrnes *et al.*, 1953); but there was no antifibromatogenic action in the guinea-pig (Mardones *et al.*, 1954b).

The procedure adopted for the study of the antiluteinizing potency of the mentioned 5 compounds was the same as in former work. Guinea-pigs were castrated and one of the ovaries was grafted into the spleen. Two weeks afterwards a pellet of estradiol and a pellet containing a mixture of the P-derivative with cholesterol were implanted subcutaneously. The use of such mixtures allows for an absorption of small quantities of the compound. Absorption per day was calculated dividing total loss of weight of the pellet by the number of days, on the assumption of non-selective absorption of the specific steroid present in the mixture (Fuenzalida and Lipschutz, 1953). If one wishes to avoid such an assumption the figures given in the fifth column of our table must be multiplied by 2.5 (experiments with 40% pellets) and by 5 (experiments with 20% pellets). The animals were necropsied at 60 days after grafting (45 days of steroid action). The spleen was fixed in Bouin's fluid and the part containing the graft was cut with a razor into thin slides; representative slides were used for microscopical examination. This procedure makes it unnecessary to cut each graft in series. The number of corpora lutea was counted. The diameter of each corpus luteum was measured; each animal was characterized by its largest corpus luteum diameter.

In former work we have calculated the average number of corpora lutea per animal in each group and the average of largest diameters in the group. For the purpose of comparison it is advisable to combine these two quotients in one (see table).

Table 1 contains the results with 89 animals. Older findings with estradiol only, with P and ET (72 animals) have been included for comparison (see groups with asterisk).

TABLE 1. 89 CASTRATED GUINEA-PIGS WITH INTRASPLENIC OVARIAN AUTOGRAFTS. PELLETS OF ESTRADIOL, OF PROGESTERONE AND OF 5 DIFFERENT PROGESTERONE DERIVATIVES HAVE BEEN IMPLANTED SUBCUTANEOUSLY.—72 EXPERIMENTS FROM FORMER WORK (*) HAVE BEEN ADDED FOR COMPARISON

Group	Progestational potency[1]	Estradiol per day, μg.	Progestational steroid per day, μg.	Number of animals		Corpora lutea			Number of pellets of progest. steroid per animal
				Total (n)	With corp. lut. (n_1)	Average per animal[2] (x)	Average of largest diameters,[3] mm. (y)	Coefficient of luteinizat. (xy)	
0*	—	26–28	0	30	27	1.50	1.60	*2.40*	0
	1		Progesterone						
Ia*		25–52	8–15 (12)	18	9	1.40	1.06	*1.48*	1/2 –1, 40%
Ib*		31–54	16–23 (20)	10	3	0.80	0.91	*0.73*	
Ic		33–74	22–58 (40)	8	0	0	0	*0*	1/2, 40%
	4–8		19-nor-Progesterone						
IIa		41–78	2–4 (3)	9	1	0.11	0.45	*0.05*	1/2, 20%
IIb		35–89	12–23 (19)	9	0	0	0	*0*	1/2, 40%
	2–3		Δ^{11}-dehydroprógesterone						
IIIa		44–73	10–13 (11)	9	3	0.44	1.70	*0.75*	1, 40%
IIIb		26–64	34–74 (42)	11	0	0	0	*0*	3, 40%
IV	ab. 1/33–1/10[4]	47–53	11-Keto-progesterone 29–55 (41)	6	2	0.50	1.23	*0.62*	1, 40%
V	ab. 1/33–1/12[4]	40–77	11β-hydroxy-progesterone 33–66 (47)	9	1	0.11	1.70	*0.19*	2, 40%
VI	>1/12[5]	30–73	11α-hydroxy-progesterone 22–63 (43)	19	14	1.40	1.50	*2.10*	1–2, 40%
	1/10		Ethinyl-testosterone						
VIIa*		34–64	150–208 (173)	10	10	1.70	1.21	*2.06*	8–12, pure
VIIb*		21–37	458–967 (728)	4	3	0.75	1.69	*1.27*	20, pure
VIII	ab. 1/33–1/10	31–80	11-Keto-progesterone 494–688 (560)	8	0	0	0	0	2, 80%

* Taken for comparison from Table 1 in Mardones *et al.*, 1951.
[1] See Tullner and Hertz, 1953; Meystre *et al.*, 1948; Ingle *et al.*, 1953; Byrnes *et al.*, 1953.
[2] Sum of Corp. lut. of the group divided by n.
[3] Sum of the largest diameters of the group divided by n_1.
[4] Various tests: (a) Astwood test (formation and maintenance of deciduoma in traumatized uterus of pseudopregnant rat)—less than 8% the activity of P; (b) copulatory reflex in estrogen-primed ovariectomized guinea-pigs—about 3% the activity of P; (c) Corner-Allen test with rabbit uterus—4 to 10% the activity of P (Byrnes, 1953).
[5] No reaction in Astwood test with the 12 fold of P (Byrnes *et al.*, 1953).

The very considerable antiluteinizing potency of 19-nor-P, compared to P, is fully evident (compare IIa with Ia). The greater antiluteinizing potency of 19-nor-P remains striking even when admitting for IIa non-selective absorption, i.e. 10 to 20 μg. per day, and comparing with Ib instead of Ia. Thus, 19-nor-P has so far been shown to be superior to P in all actions hitherto studied: progestational (Tullner and Hertz, 1953) antifibromatogenic, antihysterotrophic [antiestrogenic] (Mardones *et al.*, 1954a) and antiluteinizing.

We were greatly interested in the antiluteinizing potency of Δ^{11}-dehydro-P because its antifibromatogenic and antihysterotrophic potencies have been shown not to be superior, or only slightly superior, to that of P (Mardones *et al.*, 1953) though the compound is 2 to 3 times more progestational than P (Meystre *et al.*, 1948). Its antiluteinizing potency is apparently only slightly superior to that of P (compare IIIa with Ia).

Now let us take the C-11 derivatives of P.

11-keto-P and 11β-OH-P have only a very insignificant progestational

potency which is apparently about 10 to 30 times less than that of P (Ingle *et al.*, 1953; Byrnes, 1953). However, their antiluteinizing potency is surprisingly high. Thus, with quantities of 11-keto-P only about twice those of P the same antiluteinizing effect is obtained (compare IV with Ib). The antiluteinizing potency of 11-keto-P is not smaller, probably even greater, than that of 11β-OH-P (compare V with IV). The antiluteinizing effect with 47 μg. of 11β-OH-P per day was not much smaller than with 40 μg. of P (compare V with Ic).

The striking behaviour of these 2 compounds (11-keto-P and 11β-OH-P) becomes especially evident when comparing with the figures for ET whose progestational potency is certainly superior to that of 11-keto-P and 11β-OH-P. But the antiluteinizing potency of ET is many times less than that of the mentioned 2 P-derivatives (compare VIIa and VIIb with IV and V). To convince oneself of the fundamental difference between the 2 P-derivatives on one hand, ET on the other hand, it is worthwhile to compare also VIIb with VIII.

One may summarize the results of this comparison in the following words: the antiluteinizing potency of ET is not concomitant with its progestational potency in so far as the antiluteinizing potency of ET is *smaller* than expected according to its progestational potency (Mardones *et al.*, 1951); nor is the antiluteinizing potency of 11-keto-P and 11β-OH-P concomitant with its progestational potency in so far as the antiluteinizing potency of these 2 compounds is *greater* than expected according to their progestational potency (present work). We shall come back to these surprising results (see Discussion).

Experiments with 11α-OH-P also offer considerable interest. So far no estrogenic, androgenic or progestational potencies have been seen in the rat (Byrnes *et al.*, 1953). But the same authorities found that it has remarkable antiestrogenic (antihysterotrophic) and antiandrogenic potencies, and what is of still greater interest it inhibits the gonadotrophic action of the hypophysis as evidenced by the diminished weight of the ovaries; as to this 11α-OH-P was even more effective than P (Byrnes *et al.*, 1953). In our work with the castrated guinea-pig no antiestrogenic (antifibromatogenic) potency was discovered, even with a dose 30-fold that of the antifibromatogenic threshold of P (Mardones *et al.*, 1954b). As to this the difference between 11α-OH-P and 11β-OH-P was very striking. There was apparently a slight antihysterotrophic potency but it was certainly much inferior to that of 11β-OH-P (compare groups VI and VIII in Mardones *et al.*, 1954b; also groups VII and X in the same paper). This divergency between results obtained in the rat and the guinea-pig is impressive. But no less impressive is the fact that contrary to results in rats, no antigonadotrophic action was obtained with 11α-OH-P in the guinea-pig, as judged by experiments in which the antiluteinizing potency was tested. With 11α-OH-P there was not the slightest antiluteinizing action

when amounts were administered coincident with definitely antiluteinizing quantities of 11-keto-P or 11β-OH-P. Thus, with about 43 μg. of 11α-OH-P there was not the slightest antiluteinizing action (compare VI with O*); on the contrary a definite antiluteinizing effect was obtained with about 41 μg. of 11-keto-P per day (groups IV) or with 47 μg. of 11β-OH-P per day (groups V).

Our results with the comparative antiluteinizing action of the 3 11-oxy-derivatives of P in guinea-pigs in which no antiluteinizing effect was obtained with 11α-OH-P, are coincident with those of Ford (1954) in pregnant rats: pregnancy which depends on intact corpora lutea was not interfered with when quantities of 11α-OH-P as large as 5 mg per day were administered during the whole pregnancy.

DISCUSSION

In the Introduction we referred to the discrepancy existing between DCA and ET as to the antiluteinizing potency which is concomitant with the progestational potency in the case of DCA but not in the case of ET. The increase of the progestational activity of ET, compared with testosterone, is not concomitant with an increase of its antifibromatogenic, antihysterotrophic or antiluteinizing potencies (Iglesias and Lipschutz, 1946; Lipschutz, 1950, p. 147; Mardones *et al.*, 1951). Fundamentally different from the behaviour of ET, but coincident with that of DCA, is the behaviour of 19-nor-P. With the *decrease* of the progestational potency of DCA compared to P there is a concomitant decrease of antiestrogenic potencies (antifibromatogenic, antihysterotrophic and antiluteinizing actions). Similarly, with the *increase* of the progestational potency of 19-nor-P compared to P there is a concomitant increase of its antiestrogenic potencies (antifibromatogenic, antihysterotrophic and antiluteinizing actions). With Δ^{11}-dehydro-P which is more progestational than P, little seems to change as to its various antiestrogenic potencies; but since the increase of the progestational potency from P to Δ^{11}-dehydro-P is much less than from P to 19-nor-P, it seems impossible to obtain in the first case such a significant picture as in the second.

We ventured the idea that the understanding of the behaviour of a synthetic steroid like ET in the body might be fostered by taking into consideration that this steroid is structurally outside any normal steroid pattern. When applied directly to the endometrium of the rabbit ET is as progestational as progesterone (Höhn and Robson, 1950). But when it enters into the general circulation it is 10 times less progestational than progesterone; its antifibromatogenic or antihysterotrophic potency is apparently not greater than that of testosterone; and no antiluteinizing effect is obtained even with the 10 fold quantity of P which gives complete prevention of luteinization. Similar is the behaviour of vinyl-testosterone and ethinyl-androstenediol (Mardones *et al.*, 1951).

Why a steroid which is structurally outside any normal steroid pattern should behave in the above manner is an interesting question but a question probably out of reach of our actual knowledge.

Non-concomitancy of antiestrogenic potencies with progestational potency is met with also with P-derivatives as 11-keto-P and 11β-OH-P. But with these compounds which correspond to a normal steroid pattern in the body the course of things is again quite different than with ET. Oxidation at $C_{(11)}$ has cut down their progestational potency. However, they keep their antiestrogenic potencies: they are antifibromatogenic and antihysterotrophic though, indeed, much less than P. But the decrease of the antifibromatogenic and antihysterotrophic potency due to oxidation at $C_{(11)}$ is much less than the decrease of their progestational potency (Mardones *et al.*, 1954b). Again, as to this the findings with the comparative *antiluteinizing* potency of P and of the 2 P-derivatives are amazing: 11-keto-P and 11β-OH-P are certainly less antiluteinizing than 19-nor-P, Δ^{11}-dehydro-P and P but they are still powerful antiluteinizers!

It seems justified to ask whether use could be made of P or P-derivatives in clinical trials in which an antigonadotrophic action is aimed at. The idea that in clinical trials recourse could be made to $C_{(11)}$ derivatives of P has been forwarded by Byrnes *et al.*, (1953) when working with 11α-OH-P. However, this compound has been shown in the present work in guinea-pigs to be inferior to 11-keto-P and 11β-OH-P as to certain antigonadotrophic activities as the luteinizing one, and as already mentioned it is apparently void of any antiluteinizing potency in the rat (Ford, 1954). It is remarkable that likewise the diabetogenic potency of 11α-OH-P in partially depancreatized force-fed rats is much smaller than that of 11-keto-P and 11β-OH-P (Ingle *et al.*, 1953; also Stafford and Baker, quoted from Ingle *et al.*). Deposition of glycogen in the liver in adrenalectomized animals is greatly stimulated by corticosterone or 17-hydroxy-corticosterone which have the 11-OH in the β configuration; on the contrary, when the 11-OH is introduced in the α configuration, by the action of certain microorganisms or by chemical means, the 17-hydroxy-compound has only a very insignificant effect on glycogen deposition (Segaloff and Horwitt, 1953).

It has been assumed that 11β-OH-P might be an intermediary in the formation of corticosterone or 17-α-hydroxy-corticosterone (Hechter *et al.*, 1951); more recently P was converted in good yield by adrenocortical mitochondria into 11β-OH-P (Brownie *et al.*, 1954) though indeed the last mentioned authorities insist that "it is at present not possible to decide whether these reactions may or may not occur" in the body. On the other hand, 11α-OH-P is, as far as we can judge, outside any physiological reality.

After all this, when dealing with the question of clinical trials with antigonadotrophic compounds, one must decide whether preference shall

be given to powerful antiluteinizers such as P, Δ^{11}-dehydro-P and 19-nor-P, or to antiluteinizers much less potent such as 11-keto-P and 11β-OH-P In the first case antiluteinizing action is paramount but goes together with progestational action; in the second case antiluteinizing action is much less pronounced but scarcely accompanied by progestational action.

SUMMARY

The comparative antiluteinizing potency of progesterone derivatives has been studied in experiments with oestrogen-induced luteinization in intrasplenic ovarian autografts in castrated guinea-pigs.

The antiluteinizing potency of derivatives whose progestational action is greater than that of progesterone, increases compared to the latter. This is true especially for 19-nor-progesterone; the increase is less pronounced in the case of Δ^{11}-dehydroprogesterone.

The antiluteinizing potency of derivatives whose progestational action is much smaller than that of progesterone, diminishes compared to the latter. However, the antiluteinizing potency of compounds such as 11-keto-progesterone and 11β-hydroxyprogesterone is *greater* than expected according to their very insignificant progestational potency.

Thus non-concomitancy of the progestational and antiluteinizing potencies of 11-ketoprogesterone and 11β-hydroxyprogesterone presents itself in a different aspect than non-concomitancy formerly found with ethinyltestosterone the antiluteinizing potency of which is *smaller* than expected according to its progestational action.

The differential behaviour of the mentioned progesterone derivatives on one hand, and of ethinyltestosterone, on the other hand, is tentatively attributed to the fact that the latter compound is outside the normal steroid pattern in the body.

Likewise, no antiluteinizing action was obtained with 11α-hydroxyprogesterone, a compound also outside the normal steroid pattern in the body.

Some comparative pharmacological aspects related to the idea of clinical trials with antigonadotrophic derivatives of progesterone are also discussed.

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ANALYSIS OF THE BLOOD SUGAR RESPONSE OF OBESE-HYPERGLYCEMIC MICE AND NORMAL MICE TO HORMONES: INSULIN, GLUCAGON, AND EPINEPHRINE[1]

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SHULL and Mayer (1955) described the effects of growth hormone, corticotropin, and cortisone on the blood sugar of the obese-hyperglycemic mice and their non-obese siblings. It was of importance to assay the effects of glucagon and epinephrine on the blood sugar of the obese mice since it has been found that the turnover of liver glycogen is greatly accelerated (Shull and Mayer, 1955) and the activity of liver phosphorylase is greatly elevated (Shull, Ashmore and Mayer, 1955) in the obese-hyperglycemic mice. The insulin resistance previously reported for these mice (Mayer *et al.*, 1951) has been further analyzed by determining the blood sugar response after administration of various doses of insulin.

METHODS

The mice used in these experiments were selected from stock cages where they had been maintained *ad libitum* on commercial laboratory chow and water. As nearly as possible equal numbers of males and females were used in each assay. The mice ranged from 3 to 6 months in age. Blood sugar determinations were carried out in duplicate on 0.1 cc. samples of tail vein blood by the method of Somogyi (1945) using the colorimetric procedure of Nelson (1944). The following special methods and procedures were used in the individual experiments.

Insulin.—The animals listed in Table 1 were given by intraperitoneal injection 20 units of crystalline zinc insulin (Lilly) per kg. body wt. Blood samples were obtained at

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[3] Part of the data in this article is from a thesis presented to the Division of Medical Sciences, The Graduate School of Arts and Sciences, Harvard University, in partial fulfillment of the requirements for the degree of doctor of philosophy in the subject biochemistry.

zero and 2.5 hr. after the administration of insulin. The fed animals had access to food until insulin was administered at which time the food was taken away. The obese animals used in the fasting study were first fasted 24 hr. and then assayed with insulin. After 24 hr. of continued fasting they were reassayed with insulin.

Glucagon[4] *and Epinephrine.*—These hormones were given at a level of 25 μg./animal by intraperitoneal injection. Samples of blood for blood sugar determinations were obtained immediately before and 30 min. after administration. Glucogon was dissolved in water by adjusting the pH to 10 with dilute (0.05N) NaOH. Epinephrine in the form of the hydrochloride was dissolved in physiological saline. The carbohydrate-free diet employed has been previously described (Mayer and Jones, 1953). Animals were maintained on this diet for 2 weeks before assays were performed.

TABLE 1. BLOOD SUGAR RESPONSE OF FED AND FASTED OBESE-HYPERGLYCEMIC MICE AND THEIR NON-OBESE CONTROLS TO 20 UNITS OF INSULIN PER KG. BODY WEIGHT

	Number of animals	Body weight* g.	Dietary	Pretreatment blood sugar* (mg. %)	Posttreatment blood sugar* (mg. %)	Decrease in blood sugar* (mg. %)
Obese	4	67 ± 9	Fed	255 ± 94	125 ± 106	130 ± 32
Non-Obese	4	23 ± 2	Fed	124 ± 13	8 ± 2	117 ± 14
Obese†	5	62 ± 7	24-hr. Fast	92 ± 27	31 ± 15	61 ± 24
Obese†			48-hr. Fast	71 ± 8	25 ± 10	46 ± 9
Non-Obese	6	28 ± 3	24-hr. Fast	66 ± 17	12 ± 5	54 ± 20

* Mean ± standard deviation.
† Assayed after 24-hr. fast; without the benefit of food were reassayed 24 hr. later.

RESULTS

Table 1 lists the effects of crystalline zinc insulin on the blood sugar of fed and fasted obese and non-obese mice. When the responses of the fed obese and non-obese mice were compared, it was found that the absolute decreases in blood sugar in response to insulin were similar in the two groups. However, since the initial blood sugars were higher in the obese than in the non-obese mice, the levels 2.5 hr. after receiving insulin were also different: the blood sugar levels in the non-obese mice were extremely low, and all animals were in convulsions at the 2.5-hr. assay period. In contrast, the levels for the obese mice were in the normal range for fed non-obese mice and no untoward effects were observed. In order to eliminate in part the high pretreatment blood sugars of the obese mice, a group of these animals was fasted 24 hr. and their insulin sensitivities then assayed. Inspection of the results for these animals listed in Table 1 indicated that the posttreatment blood sugars of the fasted obese mice had now reached very low levels. However, unlike non-obese mice, this group of obese mice recovered spontaneously without the benefit of food. Fur-

[4] Lot #208-158B-197 kindly furnished by the Eli Lilly Co., Indianapolis, Ind. The insulin content of this preparation was between 0.005 and 0.05 units per mg. of glucagon. When injected intravenously into anesthetized cats a dose of 0.2 μg./kg. body wt. produced a 30 mg.% to 40 mg.% rise in blood sugar in 10 to 15 min.

thermore, when these animals were continued on their fast for an additional 24 hr. and then given an additional 20 units of insulin/kg. body wt., all of them again recovered without the benefit of food and survived an additional 24-hr. fast. In contrast to the fasted obese mice, the fasted non-obese mice displayed convulsive seizures and all animals died within 4 hr. after receiving insulin. Finally, in order to ascertain the possible influence of the method of insulin administration, 4 obese mice were given intravenously 1.2, 4.0, 8.0 and 12.0 units of insulin respectively. The obese mice survived the injection with no untoward symptoms. It may be noted incidentally that the lowest dose, 1.2 units, represented 20 units/kg. body wt. and the highest dose represented 260 units/kg. body wt. for the respective animals assayed.

The physical manifestations displayed by the insulinized obese and non-

TABLE 2. EFFECTS OF GLUCAGON AND EPINEPHRINE ON THE BLOOD SUGAR OF OBESE MICE MAINTAINED ON VARIOUS DIETARIES

	Number of animals	Dietary	Treatment	Change in blood sugar* (mg. %)
Treated	19	Chow	Glucagon	79 ± 40
Control	13	Chow	Water	0 ± 46
Treated	22	12-hr. Fast	Glucagon	197 ± 54
Control	17	12-hr. Fast	Water	46 ± 44
Treated	4	CHO-Free Fiet†	Glucagon	85 ± 65
Control	4	CHO-Free Diet†	Water	33 ± 28
Treated	8	Chow	Epinephrine	149 ± 95
Control	4	Chow	Water	8 ± 46
Treated	5	12-hr. Fast	Epinephrine	249 ± 88

* Mean ± standard deviation.
† High fat carbohydrate-free diet described by Mayer and Jones (1953).

obese mice are worthy of note. The non-obese mice, whether fed or fasted, usually showed uncoordinated muscular activities one to 2 hr. after receiving insulin and displayed convulsions with violent rigors 2 to 3 hr. after insulin administration with fatal termination usually before 4 hr. The fasted obese mice behaved much differently. At the height of their hypoglycemia they became motionless and fixed in one position and place in their cages. Complete recovery from these symptoms was usually observed in 4 hr. Three of the fasted obese mice treated with insulin showed blood sugars of 17 mg.%, 13 mg.%, and 9 mg.% respectively. These values were within one standard deviation of the mean blood sugar level of the fasted non-obese mice and indicated that some of the obese mice were subjected to hypoglycemias comparable to those of the non-obese mice.

The effects of glucagon and epinephrine on the blood sugar of obese mice are shown in Table 2. When the changes in blood sugar in response to glucagon for animals maintained on carbohydrate-containing chow diets and carbohydrate-free diets were compared, the responses were found to be very similar. This indicated that intake of carbohydrate as

such was not necessary for glucagon to induce hyperglycemia in these animals. When the blood sugar responses of the fed animals were compared to the response of fasted animals, a highly significant difference was found ($P<0.01$). The blood sugar of the fasted group was increased 197 mg.% while that of the chow-fed group was increased by only 79 mg.%. Such a finding was surprising since glucagon presumably produces hyperglycemia by accelerating the breakdown of liver glycogen which is decreased under fasting conditions. A similar dose of epinephrine also produced a greater hyperglycemic response in fasted obese animlas than it did in fed obese animals. Table 2 shows that epinephrine-treated, like glucagon-treated fasted obese mice, displayed an increase in blood sugar approximately 100 mg.% greater than their fed counterparts. For the dose used, the blood sugar response to epinephrine in both fed and fasted obese-hyperglycemic

TABLE 3. EFFECTS OF GLUCAGON AND EPINEPHRINE ON THE BLOOD SUGAR OF FED AND FASTED NON-OBESE MICE

	Number of animals	Dietary	Treatment	Change in blood sugar* (mg. %)
Treated	13	Chow	Glucagon	44 ± 33
Control	13	Chow	Water	18 ± 22
Treated	8	12-hr. Fast	Glucagon	7 ± 22
Control	3	12-hr. Fast	Water	−16 ± 37
Treated	10	Chow	Epinephrine	189 ± 77
Control	4	Chow	Water	11 ± 16
Treated	5	12-hr. Fast	Epinephrine	119 ± 26

* Mean ± standard deviation.

mice was somewhat greater than the blood sugar response to glucagon in the fed and fasted mice respectively.

The effects of glucagon and epinephrine on the blood sugar of non-obese control mice are listed in Table 3. Glucagon caused a small increase in the blood sugar of the fed animals, but after a 12-hr. fast it produced no significant increase in the blood sugar of the non-obese mice. The comparison studies performed with similar doses of epinephrine indicated that the fed non-obese mice were much more sensitive to the hyperglycemic effects of epinephrine than to those of glucagon. In contrast to the obese mice, the fasted non-obese control mice displayed smaller hyperglycemic responses to both glucagon and epinephrine than fed animals.

DISCUSSION

The extreme insulin resistance displayed by the obese mice was, in addition to their diabetes, one of the most unusual characteristics of their obese-hyperglycemic syndrome. It differed from the clinical connotation of insulin resistance (Conn, 1953) in that the blood sugar decreased in response to insulin, in some cases to levels comparable to those seen in the control animals, but apparently did not remain at low levels long enough

to prove fatal to the obese animals. Convulsions were not observed in the obese mice. A possible inability to display convulsive muscular movements could be ruled out since obese mice treated with large doses of sodium salicylate show convulsive movements similar to those of the insulinized controls. When treated with insulin the non-obese mice invariably died unless exogenous glucose was supplied them. The insulin resistance was not overcome in the obese mice by additional insulin and fasting treatments, by larger amounts of insulin, nor by intravenous administration of this hormone. The insulin resistance of the obese mice differs from the hereditary insulin resistance of a highly inbred strain (KL) of mice described by Chase *et al.* (1948). At 30–40 days of age mice of the KL strain were able to tolerate 300 units of insulin. However, subsequent work by MacGrath and Snedecor (1953) on this strain of mice indicated that these animals do not display characteristic resistance when the dose of insulin is low, i.e., one to 2 units. The insulin resistance of the obese mice is also different from the resistance to this hormone displayed by the albino rat. The albino rat has been reported to tolerate 500 units of insulin/kg. body wt. if allowed free access to food (McIntyre and Burke, 1937). The obese mice display their resistance even when fasted and not allowed access to food.

The hyperglycemic effects of glucagon and epinephrine were assayed in both the obese and non-obese mice. Comparable doses of glucagon produced greater blood sugar increases in both the fed and fasted obese mice than in the respective fed and fasted non-obese mice. In the latter group fasting completely abolished the hyperglycemic effects of this hormone while in the obese mice it caused a much greater hyperglycemic response than in fed animals. The hyperglycemic response to administered epinephrine was similar to that of glucagon in both fed and fasted obese mice.

The marked differences in response of fasted obese-hyperglycemic mice treated with glucagon compared to the response of these fasted mice treated with growth hormone was of interest. As was previously shown, growth hormone caused no increase in blood sugar of fasted obese animals (Shull and Mayer, 1955). One possibility is that the hyperglycemic effect of growth hormone was not mediated through the tropic release of glucagon in these animals (Bornstein *et al.*, 1951). However, another possibility is that the dose of growth hormone employed may not have been large enough to have caused the release of sufficient glucagon to produce hyperglycemia (Wrenshall *et al.*, 1956).

The failure of glucagon to produce an increase in the blood sugar of the fasted non-obese mice while epinephrine produced an increase was of interest. Although both hormones produce hyperglycemia by the same mechanism, this was one situation in which epinephrine produced a hyperglycemic response while glucagon did not. Root *et al.* (1953) reported an increase of approximately 60 mg.% in the blood sugar of white mice

fasted 17 to 18 hr. which had been injected subcutaneously 30 min. before with 2 μg. of crystalline glucagon. They observed a maximum increase in 15 min., and significant elevation persisted through the one-hr. assay period. These results were much different from those presented here for the fasted non-obese mice. They perhaps represent the difference between a highly inbred strain of mice such as those used by Root *et al.* and a very heterogeneous stock of mice such as the non-obese mice used in the present studies. Since the two groups of mice employed here were composed of littermates and assayed under identical conditions, it was felt that a real difference existed between the hyperglycemic effects of glucagon in the obese and non-obese mice. The possible etiological significance of this difference will be discussed in later publications.

SUMMARY

The obese-hyperglycemic mice showed a hypoglycemic response to administered insulin comparable in magnitude to that displayed by their insulinized non-obese controls. However, the obese mice were not killed by insulin even when given repeated doses under fasting conditions. The non-obese control animals when treated under similar conditions with insulin were always killed by this hormone.

The fed obese-hyperglycemic mice displayed hyperglycemic responses of 79 mg.% and 149 mg.% respectively to the parenteral administration of 25 μg. of glucagon or epinephrine. After a 12-hr. fast glucagon and epinephrine produced 197 mg.% and 249 mg.% increases respectively in the blood sugar of the obese mice. In contrast glucagon produced a small increase in blood sugar in the fed non-obese controls which was completely abolished by fasting. Epinephrine caused a marked hyperglycemic response in the fed non-obese mice; the hyperglycemia produced by this hormone was significantly decreased under fasting conditions.

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CASTRATION AND THE GROWTH OF MUSCLES IN THE RAT[1]

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THE endocrine secretions of the testis influence the growth of certain muscles of the guinea pig out of proportion to the body weight (Papanicolaou and Falk, 1938; Kochakian, Humm and Bartlett, 1948; Scow and Roe, 1953; Kochakian *et al.*, unpublished). Since this phenomenon is an expression of the protein anabolic effect of androgens (Kochakian, 1946), it seemed that a similar phenomenon might be evident in the rat (Kochakian, 1950; Kochakian *et al.*, 1950).

PROCEDURE

Rats of the Sprague-Dawley strain were castrated at 36–40 days of age. They were maintained in individual cages in an air-conditioned room kept at 25.5–26.6°C. and with constant artificial light 12 hours per day. They were fed *ad libitum* a prepared mixture in parts per 100 consisting of casein 16.7, yeast (Fleischmann's 2019) 9.2, sucrose 61.2, hydrogenated vegetable oil 7.4, celluflour 1.8, and Wesson's salts 3.7. In addition a daily supplement of one drop of cod liver oil (Patch's) and one drop of 34% tocopherol concentrate[2] from wheat germ oil diluted ten-fold with Wesson oil was mixed with the food.

The rats were autopsied at 52, 94, 96, and 132 days after castration. The internal organs were weighed and the remaining viscera and abdominal fat were discarded. The carcass with the skin intact was weighed, placed in a plastic bag and frozen for later removal of the muscles from both sides. The muscles were dried to constant weight in an oven at 90–95°C. for 48 hours. Weights were determined with appropriate Roller-Smith torsion balances. The nitrogen was determined in duplicate by the micro-Kjeldahl method after dissolving the dried muscles in alcoholic potassium hydroxide (Kochakian and Stettner, 1948; Kochakian *et al.*, unpublished).

RESULTS

Organs: The well known changes were evident (Table 1) as a result of castration. The seminal vesicles and prostates almost completely dis-

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[1] This investigation was aided under contract AT(40-1)1741 with the U. S. Atomic Energy Commission, by grant C-1954 of the National Cancer Institute of the United States Public Health Service and an Institutional Grant from the American Cancer Society.

[2] The tocopherol concentrate was generously provided by the Distillation Products Incorporated through the courtesy of Dr. P. L. Harris.

appeared, the kidneys were significantly reduced in size and a suggestive decrease in the liver occurred. On the other hand, the thymus significantly increased and the adrenals showed a suggestive increase.

Muscles: The castrated rats showed a slower rate of body weight increase and a slightly lower final body weight. The changes in muscle mass in general followed the growth of the body (Fig. 1). The 29 different examples of skeletal muscle and the heart and diaphragm (Table 2) showed no striking or significant changes in mass or nitrogen concentration as a result of castration. Furthermore, three other studies which are summarized (Table 3) with the above study (Table 2) also indicated a failure of castration to effect the growth of specific muscles out of proportion with the body weight.

The duration of castration from 52 to 113 days (Table 3, Fig. 1) did not influence the results.

TABLE 1. CHANGE IN ORGAN WEIGHTS AFTER CASTRATION IN THE RAT[1]

Organ	Normal, gm.	Castrate, % diff.	Organ	Normal, gm.	Castrate, % diff.
Sem. Ves. and Pros	2.330	−96.0	Thymus	0.360	+59.5
Kidneys	2.490	−33.8	Adrenals	0.053	+15.1
Liver	9.540	−12.3	Spleen	0.650	+ 7.9
Skin	84.0	− 7.1	Lungs	1.540	+ 3.7

[1] Castration at 40 days of age. Autopsy 96 days later.

DISCUSSION

The rat in contrast to the guinea pig does not respond to castration by disproportionate decreases in the size of specific skeletal muscles. The contrast is specially noteworthy in the head muscles which are reduced to about one-fourth the normal size in the guinea pig after removal of the testis (Papanicolaou and Falk, 1938; Kochakian *et al.*, 1950; Scow and Roe, 1953) while the changes in the rat are only in proportion with the changes in body weight. On the other hand, the marked cessation of growth of the levator ani after castration is unique for the perineal musculature as indicated by Wainman and Shipounoff, 1941.

Preliminary experiments (unpublished) indicate that testosterone propionate and methyl testosterone do not produce any remarkable disproportionate increases in any of the skeletal muscles but small increases in several of the muscles were suggested.

It is noteworthy that the kidney of the rat decreases in size and the thymus immediately increases, while the size of the kidney of the guinea pig is not altered and the thymus does not respond to castration until the guinea pig is approximately 8 months of age (Kochakian *et al.*, unpublished).

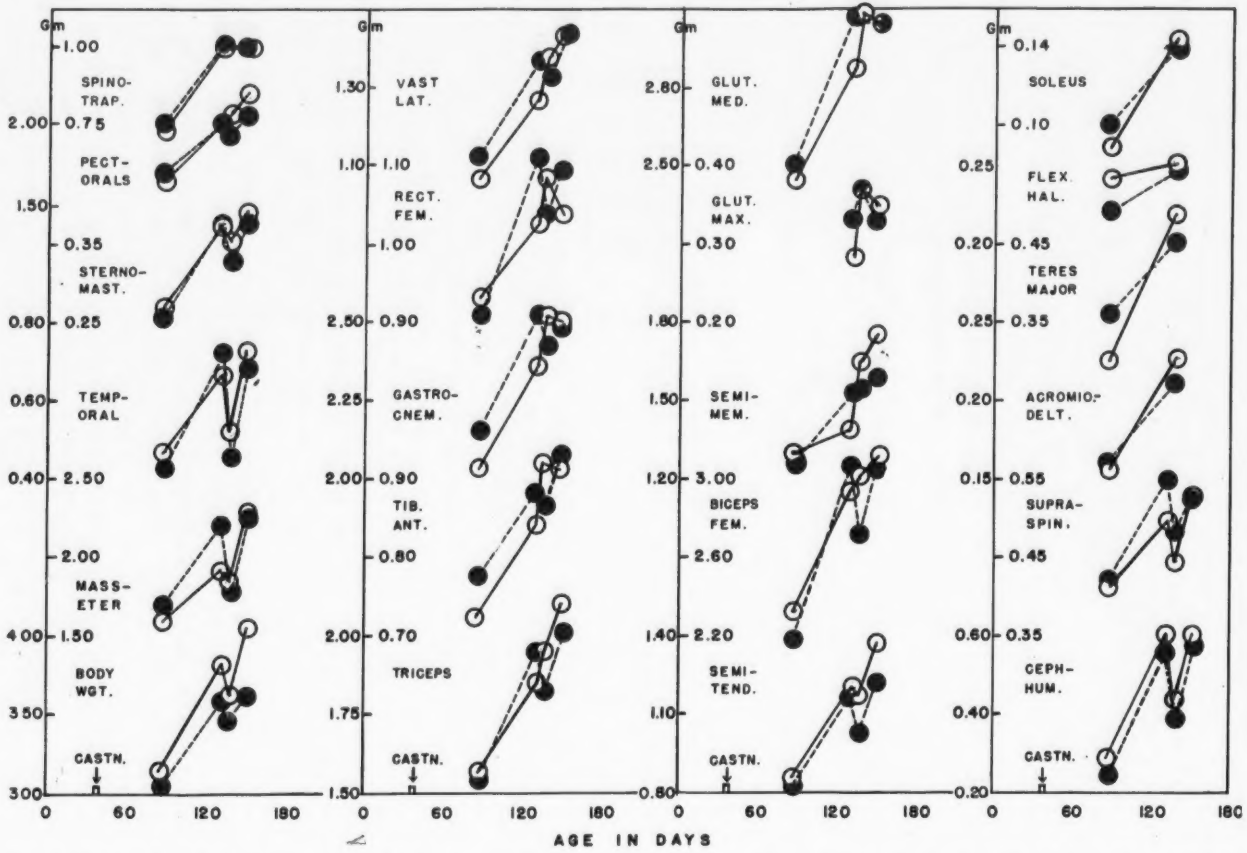

FIG. 1. Comparison between muscle weights of normal and castrated rats at different ages. Castration 36–40 days of age. Solid line ○—— ○ represents normal rats and broken line ●– – – ● represents castrated rats.

TABLE 2. COMPARISON OF THE VARIOUS MUSCLES OF THE NORMAL AND CASTRATED RAT (6 PER GROUP)[1]

	Wet weight		Nitrogen—Dry wgt.	
	Normal, gm.	Castrate, % diff.	Normal, %	Castrate, %
Body Weight	361	− 4.1		
Carcass Weight	293	− 0.7		
	Head and Neck			
Digastric	0.313	− 4.1	12.0	12.2
Masseter	1.848	− 4.1	12.7	12.8
Temporal	0.520	−13.9	12.9	12.6
Sternomastoid	0.348	− 6.0	12.3	12.8
	Chest			
Pectorals	2.071	− 7.2	12.4	12.4
	Shoulder and Back			
Acromiotrapezius	0.692	− 2.9	12.3	12.1
Spinotrapezius	0.263	− 0.7	11.4	11.4
Latissimus dorsi	1.231	− 1.4	12.5	12.2
	Scapula			
Teres major	0.486	− 7.2	13.2	12.5
Supraspinatus	0.437	+ 9.9	12.3	12.4
Acromiodeltoid	0.227	− 7.5	13.0	12.9
	Thorax			
Heart	1.020	− 6.9		
Diaphragm	1.294	0.0		
	Body Wall and Spine			
Obliques	17.133	− 8.7		
Quadratus lumborum	5.028	+ 8.7	12.9	13.2
Psoas major and minor	3.303	−13.0	11.6	11.6
	Forearm			
Triceps	1.960	− 7.4	12.8	12.7
Cephalohumerus	0.409	− 4.9	12.4	12.2
	Hip			
Gluteus medius	3.073	− 0.7	12.3	12.1
Gluteus maximus	0.369	0.0	12.5	12.0
Caudofemoralis	0.377	− 8.2	12.4	12.0
Semimembranosus	1.637	− 6.2	13.1	13.0
Semitendinosus	1.167	−12.8	12.3	11.6
Biceps femoris	3.012	−10.0	11.0	11.2
	Thigh			
Rectus femoris	1.085	− 4.6	12.7	12.8
Vastus lateralis	1.368	− 3.9	13.0	13.3
Gracilis	0.488	−10.6	11.5	11.0
	Shank			
Flexor hallucis longus	0.251	− 1.6	12.3	12.5
Tibialis anterior	0.921	− 6.1	12.9	13.0
Soleus	0.145	− 2.7	10.7	10.7
Gastrocnemius	2.523	− 4.0	12.8	11.9

[1] See Table 3 for details.

TABLE 3. SUMMARY OF A COMPARISON OF THE MUSCLE WEIGHTS OF CASTRATED AND NORMAL RATS

Experiment	I	II	III	IV	Average
Rats per Group	6	5	6	5	
Age at Castration—days	36	38	40	38	
Duration of Castration—days	52	94	96	113	
Body Weight, Normal—gm.	315	382	361	406	
			CHANGE FROM NORMAL		
	%	%	%	%	%
Body Weight	− 2.9	− 6.0	− 4.1	−10.6	−5.9
Carcass Weight	0.0	− 4.8	− 0.7	− 9.1	−3.7
			Head and Neck		
Digastric			− 4.1		
Masseter	+ 5.9	+14.7	− 4.1	− 0.9	+3.9
Temporal	− 7.3	+ 7.5	−13.9	− 6.6	−5.1
Sternomastoid	− 6.0	− 2.8	− 3.7	+ 1.6	−2.7
			Shoulder and Back		
Acromiotrapezius			− 2.9		
Spinotrapezius	+ 3.5	+ 5.1	− 0.7	− 1.9	+1.5
Latissimus dorsi			− 1.4		
			Scapula		
Teres major	+22.0		− 7.2		+7.4
Supraspinatus	+ 2.4	+10.6	+ 9.9	0.0	+5.7
Acromiodeltoid	+ 3.9		− 7.5		−1.8
			Thorax		
Heart	− 3.4	−12.2	− 6.9	−11.4	−8.5
Diaphragm	+ 3.8		0.0		+1.9
			Body Wall and Spine		
Obliques	− 8.7				
Quadratus lumborum	+ 8.7				
Psoas major and minor	−13.0				
			Forearm		
Triceps	− 2.5	+ 5.4	− 7.4	− 4.3	−2.2
Cephalohumerus	− 5.9	− 8.1	− 4.9	− 5.8	−6.2
			Hip		
Gluteus medius	− 2.5	+ 7.3	− 0.7	0.0	−1.0
Gluteus maximus			0.0	− 5.5	−2.8
Caudofemoralis		− 8.2			
Semimembranosus	− 2.3	+10.0	− 6.2	−10.7	−2.3
Semitendinosus	− 2.9	− 3.3	−12.8	−12.0	−7.8
Biceps femoris	− 5.3	+ 4.8	−10.0	− 2.5	−5.7
			Thigh		
Rectus femoris	− 2.6	+ 7.7	− 4.6	+ 5.3	+1.5
Vastus lateralis	+ 5.9	+ 7.9	− 3.9	− 0.0	+2.5
Gracilis				−10.6	
			Shank		
Tibialis anterior	+ 7.2	+ 4.8	− 6.1	+ 1.8	+1.9
Flexor hallucis longus	− 8.8		− 1.6		−5.2
Soleus	+10.0		− 2.7		+3.7
Gastrocnemius	+ 5.2	+ 7.7	− 4.0	− 1.2	+1.9
			Chest		
Pectorals	+ 3.3	+ 0.4	− 7.2	− 7.2	−2.7

SUMMARY

Rats of the Sprague-Dawley strain were castrated at 36–40 days of age and autopsied 52, 94, 96 and 132 days later. The growth of 29 skeletal muscles and the heart and diaphragm occurred at the same rate as in normal controls and in proportion to the increase in body weight. The nitrogen (protein) content of the muscles was unaltered.

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LIVER GLYCOGEN RESERVES IN EXPERIMENTAL DIABETIC KETOSIS

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THE relationship of liver glycogen to the ketosis of diabetes is not clear. The most widely held belief, cited often in medical literature, is that ketosis is a sequel to the depletion of liver glycogen reserves (Mirsky, 1942; Korenberg, 1943; McKay, 1943). According to this view the diabetic animal becomes depleted of liver glycogen because of lack of insulin, and approximates the condition of the normal animal when fasted. The depletion of hepatic glycogen reserve is thought to stimulate overproduction of ketone bodies. It is generally assumed that ketosis occurs in diabetes only after liver glycogen is exhausted. This assumption is not warranted without reexamination.

Bondy *et al.* (1948) found high liver glycogens in some humans with severe diabetic ketosis. Vallance-Owen (1952) found normal to high glycogen in the livers of patients who had died in diabetic coma without insulin therapy. Since the use of alloxan there have been reports of low, normal, or high liver glycogen in diabetic animals (Hard, 1944; Tuerkischer, 1947; Kaplan, 1945; Weber, 1946; Lackey, 1944; Renold, 1953). In general normal or moderately low values have been reported in fed animals and elevated levels in fasted diabetic animals compared to fasted normal controls. Lackey (1944) reported elevated ketone levels with somewhat diminished, but by no means exhausted, liver glycogen.

Because of persistent confusion about the status of liver glycogen in diabetic ketosis, liver glycogen and blood ketone levels were estimated in alloxanized rats.

METHODS

Male Wistar rats weighing 180–240 gm. were used. They were fed Purina Chow and maintained at constant temperature. The animals were made diabetic by the intravenous injection of alloxan mono-hydrate 4.5 mg./100 gm. They were sacrificed 10 days to 3 months later. Glycogens were done by a modification of the method of Good, Kramer and Somogyi (1933), glucose by the method of Nelson (1944) and ketone analyses by a modification of the method of Michaels (1951). The duration of fasting was 24 hours in all cases.

RESULTS

Results are summarized in Table 1. The liver glycogen of fed diabetic rats was about $\frac{1}{3}$ that of normal controls. On fasting there was no decline

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of liver glycogen although the blood glucose was halved. The normal rats on the other hand, demonstrated the usual profound drop in liver glycogen after a 24-hour fast.

The blood ketone level in the diabetic animals was elevated 10–15 fold as compared to normal controls. On fasting the ketone level of the diabetics fell somewhat while that of the normal rats rose so that similar values were approached by both groups. Whether fed or fasted the diabetic rat had elevated blood ketones coexisting with substantial liver glycogen reserves, amounting to 2% of the wet weight of the liver. This contrasted sharply with the normal animals which showed elevation of ketones concomitant with very low liver glycogen following a 24-hour fast.

TABLE 1

	Liver glycogen, %		Blood glucose, Mg. %		Blood ketone, Mg. %	
	Fed	Fast†	Fed	Fast	Fed	Fast
Normal (7)	6.49 ± .36*	(8) 0.12 ± .05	107 ± 3	78 ± 6	1.19 ± .13	7.96 ± 1.2
Diabetic (11)	1.81 ± .28	(17) 2.16 ± .29	401 ± 13	177 ± 28	17.4 ± 4.2	8.08 ± 2.8

( ) Number of animals.
* S.E. of the means.
† 24 hours duration.

DISCUSSION

It seems clear that the diabetic animal or man does not usually show profound hepatic glycogen depletion. Normal levels in fed diabetic rats and rabbits are reported by Hard (1944). Moderately decreased liver glycogen is reported by Kaplan (1945), Lackey (1944), Weber (1946) and others. Similar reduction was seen in the present experiment. In human diabetes Hildes *et al.* (1948) estimated liver glycogen by analyzing needle biopsy fragments. They could demonstrate no significant difference between diabetics and normal controls.

Despite the results of these and other investigations it might be said that since ketosis depends on exhaustion of liver glycogen, diabetic animals without glycogen depletion must not be ketotic. The concept of a causal relation of hepatic deglycogenation to ketone body over-production has been widely held (Mirsky, 1942; Korenberg, 1943; McKay, 1943). It is exemplified by the statement (Mirsky, 1942) "the most important cause of diabetic ketosis and hence of subsequent acidosis and coma is the impoverishment of the liver with respect to glycogen." However, this belief is not well substantiated. Russell (1951) points out that critical data are lacking in the literature to prove that insulin lack need be equated with deglycogenation of the liver. Tuerkischer (1947) noted that liver glycogen did not fall in his diabetic rats until they had reached terminal stages of coma. He concluded that this fall was a symptom of coma rather than of diabetes per se. Vallance-Owen (1952) histologically estimated glycogen to be abundantly present in the livers of

humans dying in diabetic coma without insulin therapy. He felt that this indicated a basic difference between human and experimental diabetes since he accepted the unproved assumption that experimental diabetic ketosis is always associated with profound depletion of liver glycogen. The study of Bondy *et al.* (1949) also indicates that severe ketosis can coexist with ample liver glycogen in the human diabetic. They concluded that the usual glycogen depletion was secondary to other aspects of diabetic coma such as inanition, vomiting, and circulatory failure.

The present experimental studies confirm the finding of Weber (1946), Tuerkischer (1947), and Renold (1953), that the liver glycogen of diabetic rats does not normally fall on fasting. Furthermore, it was noted that the relative abundance of carbohydrate stored as liver glycogen did not prevent the ketosis of the diabetic rat. This glycogen cannot be considered to be unavailable to the animal since it has been shown to disappear on exercise (Tuerkischer, 1946). It appears therefore, that the development of ketosis in diabetes is determined by other factors as well as, or instead of, liver glycogen stores.

SUMMARY

Rats were made diabetic with alloxan following which blood ketone and liver glycogen levels were estimated.

Diabetic rats, both fed and fasted, showed hyperketonemia without depletion of liver glycogen.

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A METHOD FOR IODIDE BALANCE STUDIES IN ANIMALS ON LOW IODIDE DIETS[1,2]

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RATS fed a low iodide diet until goiters developed utilized so little iodide that chemical methods were unsatisfactory for determinations of plasma PBI or of urinary and fecal iodide. A method was developed (Van Middlesworth, 1952) which involved feeding rats an I^{131}-tagged diet until an equilibrium was established between the specific activity of the diet and the specific activity of all iodide pools of the animal. Then the I^{131} in any pool or compound of the animal was assumed to be proportional to the amount of chemical iodine in that pool, regardless of the relative rates of labeling.

METHODS

Young adult male Long-Evans rats were used throughout these investigations. The animals were maintained for 4–6 months on a low iodide, goiter-producing diet (Van Middlesworth, 1955) before they were used in these experiments.

Since I^{131} has a half-life of 8 days and it frequently required several half-lives to establish equilibrium, the diet was tagged each day with an amount of I^{131} which would make each day's dose the same if corrected back to the first day that I^{131} was fed. This was accomplished by adding a fixed volume of a prepared solution of I^{131} to each rat's measured daily diet. This stock solution contained 0.1 μc. I^{131}/cc. the first day of the experiment and each day 0.5 cc. was added to the diet of each rat. As the I^{131} accumulated within the rats, the radioactive decay significantly reduced the amount of radiation. Therefore, even if the animals were to accumulate 100% of the I^{131} each day, the maximum possible retention would be 0.21 μc. and this would occur on approximately the tenth day (see Table 1). Since 100% retention never occurred, the maximum radiation was always less than 0.21 μc.

The specific activity (radioactive iodide per gram of chemical iodide) was determined by repeated analyses of the low iodide diet (Van Middlesworth *et al.*, 1953) and in another series of animals by determination of chemical iodide in the thyroid glands after equilibrium (Van Middlesworth and Truemper, 1955). From these values and the I^{131} determinations, it was assumed that the chemical iodide of any part of the rat could be determined at equilibrium. Occasionally, there were fluctuations in the iodide content of the diet, but by purchasing 100 to 150 lbs. of diet at a time, each experimental group was kept on diet with the same control number of the manufacturer throughout each experiment.

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[2] This work was carried out with the aid of grants from the United States Atomic Energy Commission and the United States Public Health Service.

TABLE 1. MAXIMUM AMOUNT OF I^{131} IN AN ANIMAL FED TAGGED DIET AS DESCRIBED (assume 100% retention of all I^{131})

Time after beginning I^{131} (days)	Maximum I^{131} accumulation, if half-life neglected (μc.)	Radioactive decay factor	Maximum I^{131} accumulation, reduced by decay (μc.)
1	0.05	0.92	0.046
5	0.25	0.65	0.16
10	0.50	0.42	0.21
15	0.75	0.27	0.20
20	1.00	0.18	0.18

For iodide balance studies the rats were placed in metabolism cages; urine and feces were collected each day and the I^{131} content was determined by a scintillation counter and compared to a counting standard prepared from one daily dose of the I^{131} solution. The results were therefore expressed as per cent of daily intake in urine and feces. The rats were in positive I^{131} balance as long as the total excreted I^{131} was less than 100% of the daily dietary I^{131}. These daily differences were added and, at equilibrium, this sum or accumulation retention measured the total functional iodine pools within the animal.

To illustrate the application of I^{131}-tagged diet in the study of iodide metabolism, the following experiments have been described:

1. Rats maintained on a low iodide diet. I^{131} was added to this daily diet and the iodide excretion was followed for 30 days (Fig. 1).

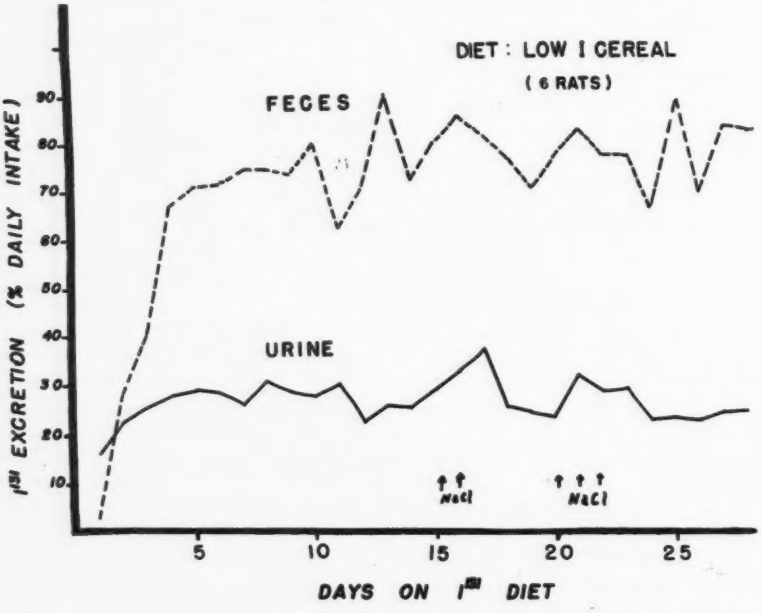

FIG. 1. Daily excretion of I^{131}, beginning the day the radioactive tag was added. This diet contained approximately, 0.015 μg. iodine per gm. At the points marked NaCl, 10% sodium chloride was added to the diet to alter the iodide excretion.

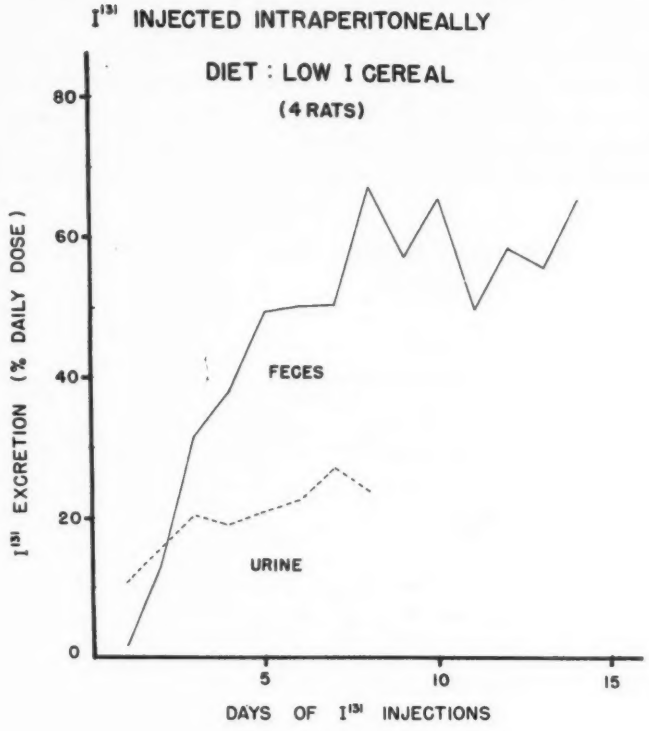

FIG. 2

2. Rats fed the same low iodide diet as group 1, but the I^{131} was given intraperitoneally twice a day (Fig. 2).

3. Iodide excretion studies on rats fed the low iodide diet but made hypothyroid by I^{131} administered 8 months previously (Fig. 3).

4. Iodide balance was followed in another group of rats for 23 days, until they were in equilibrium, and the following experiments performed (Figs. 4, 5).

4a. Thyroxine was added to their daily diet for 8 days.

4b. Recovery from thyroxine feeding was followed for 9 days.

4c. Triiodothyronine[3] was added to the daily diet, in increasing quantities for 10 days.

4d. Recovery from triiodothyronine was followed for 28 days.

5. Iodide excretion and balance study on rats fed the low iodide diet. After changes in daily urinary and fecal excretions were small, the animals were considered to be near equilibrium. Then the following experiments were performed (Figs. 5, 6).

5a. NaI was added to the diet for 15 days.

5b. The animals were permitted to recover from the NaI for 22 days.

5c. Propylthiouracil was added to the diet for 8 days.

5d. Recovery from the propylthiouracil was followed for 19 days.

[3] Triiodothyronine was supplied through the courtesy of Smith, Kline and French Co., Philadelphia, Pa.

RESULTS

Figure 1 shows that the iodide was excreted chiefly via the feces in rats fed a diet very low in iodide. Fecal iodide did not represent poor absorption since the same results were obtained (Fig. 2) when the rats daily received radioactive iodine[4] parenterally. Figure 3 shows that the I^{131} was absorbed in thyroidectomized rats and the excretion was almost entirely in the urine.

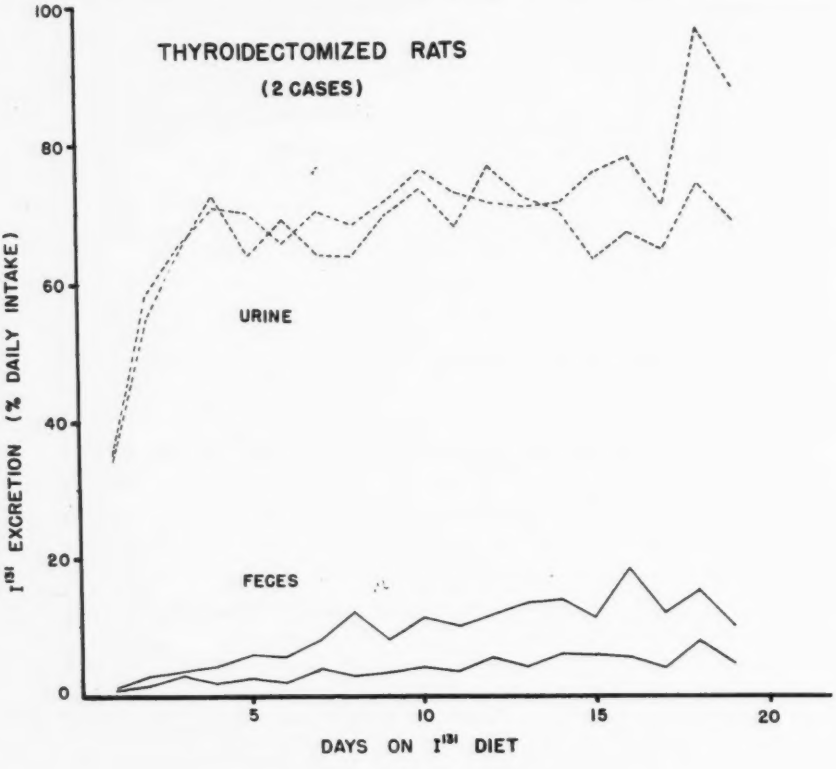

FIG. 3

A fraction of the I^{131} recovered in the feces probably resulted from urine I^{131} incompletely separated.

The effects of thyroxine and of triiodothyronine were studied by this method in goitrous rats. Thyroxine (0.5 μg/day) was added to the diet of rats equilibrated on a low iodide tagged diet (see Fig. 4). After 7 days of thyroxine there was an increase in urinary I^{131}. After 10 days, the thyroxine administration was discontinued and the animals permitted to return to their previous equilibrium. On the 41st day, triiodothyronine (0.55 μg./day,

[4] The radioactive iodine was obtained from the United States Atomic Energy Commission, Oak Ridge, Tenn.

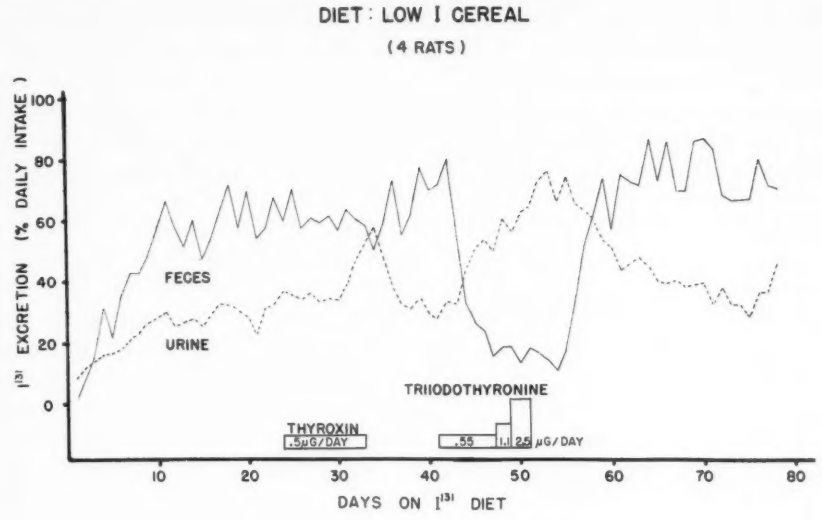

FIG. 4

equal to the iodine content of 0.5 μg. thyroxine) was added to the diet. There was a rapid and severe alteration in the iodide metabolism (Fig. 4). Within 2 days the fecal I^{131} was less than the urinary I^{131} and there was positive I^{131} balance (Fig. 5). Triiodothyronine was increased to 2.5 μg./day

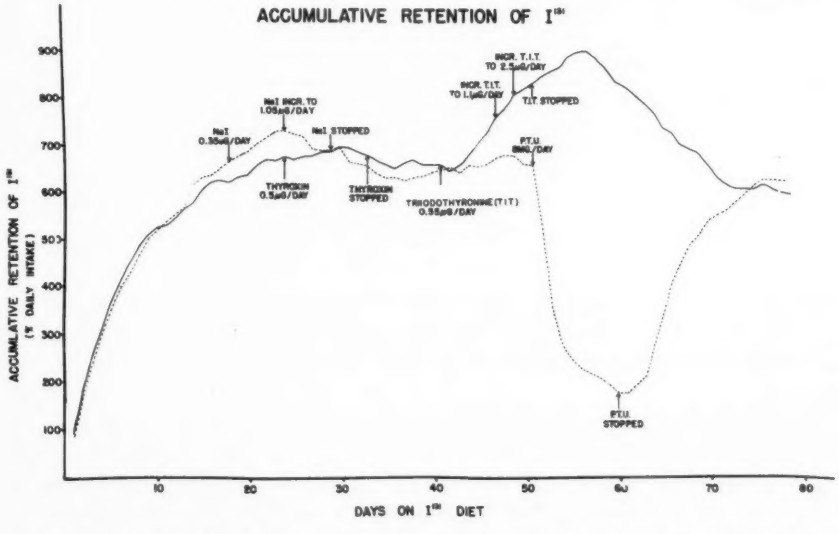

FIG. 5. The sum of the daily urine and fecal I^{131} subtracted from 100 and the differences added successively. The continuous line was drawn from Fig. 4 and the dotted line drawn from Fig. 6.

and the I^{131} balance remained positive (Fig. 5). The fecal I^{131} remained approximately 10% of the intake (Fig. 4) and the balance remained positive (Fig. 5) for 4 days after discontinuing triiodothyronine. Then the balance became negative for the next 20 days. Ultimately, the average total body I^{131} was 560% of the daily dietary I^{131}. These animals ingested 15 gm. of diet per day which contained 0.3 μg. of iodide (Van Middlesworth and

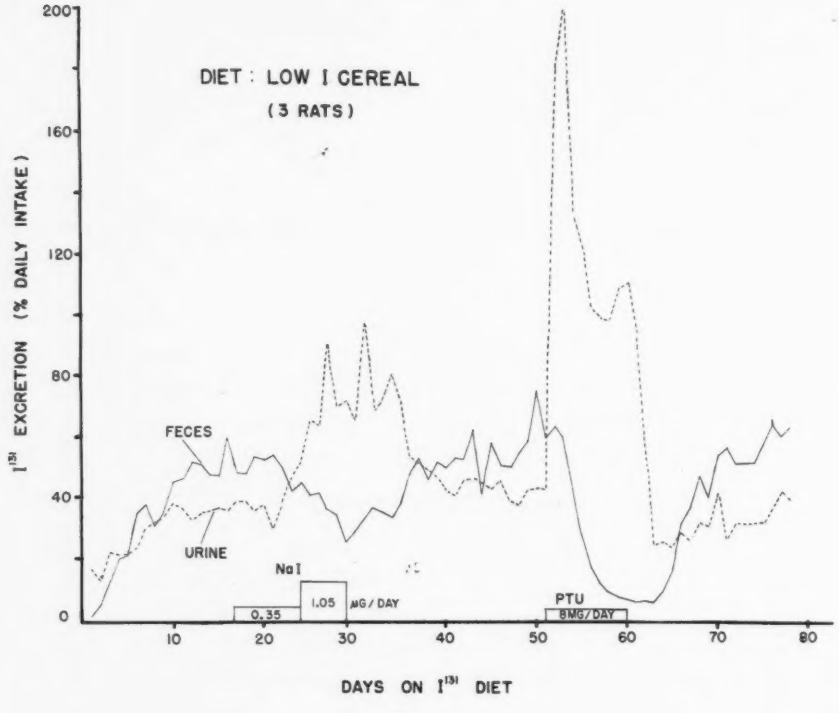

FIG. 6

Truemper, 1955), therefore, their total body iodide was 5.6×0.3 μg. $= 1.7$ μg. The thyroids averaged 70 mg./100 gm. body weight. The I^{131} content of the glands averaged 414% of the daily I^{131} intake or 1.2 μg. (70% of the body I^{131}).

The effect of sodium iodide and of propylthiouracil added to the low iodide diet (Fig. 6) was studied in the fifth group of rats. As the I^{131} excretion approached a plateau, NaI (0.35 μg./day) was added to the diet. After 6 days the relative fecal I^{131} began to decrease and urinary I^{131} increased. The dietary NaI was elevated to 1.05 μg /day and 80% of the daily I^{131} was excreted in the urine. NaI was discontinued for 20 days and the iodide excretion returned to equilibrium. On the fifty-first day, propyl-

thiouracil (8 mg./day) was added to the diet. The urinary excretion during the next 2 days was 170 and 200% respectively, of the daily I^{131} intake and the fecal I^{131} was reduced progressively to approximately 10% of the daily intake.

The I^{131} balance of these animals was negative (Fig. 5) and the total body I^{131} was reduced to 170% of the daily intake within 9 days. The I^{131} balance was positive for 14 days after discontinuing the propylthiouracil and the total body I^{131} ultimately reached a plateau at 610% of the daily intake. The thyroids of these rats contained an average of 469% of the daily I^{131} or 75% of the total body I^{131}. The thyroids of these rats, like those of group 4 (Fig. 4), averaged 73 mg./100 gm. body weight. Assuming that the specific activity of the diet (100% of daily $I^{131} = 0.3$ μg.) was the same as the specific activity of the thyroid of these rats, their thyroids contained 469% of daily $I^{131} = 1.4$ μg. of iodine.

The plasma conversion ratios were determined when the rats of group 4 and 5 were sacrificed. The trichloracetic acid precipitate averaged 95% of the total plasma I^{131} and the total I^{131} per 1.0 cc. plasma was 1.5% of the daily I^{131}. These values were too small to determine chemically but assuming equilibrated I^{131}, the chemical PBI should have been 0.4 μg%. All estimates of chemical iodine from radioactive iodine are based upon the assumption that the diet consistently contained 0.3 μg. iodide per 15 gm. The greatest variations from this that we have observed were in containers bearing different serial numbers; the minimum was 0.2 μg. iodine/15 gm. and the maximum was 0.5 μg. iodide/15 gm.

DISCUSSION

The dietary I^{131} and the functional iodide pools of the animal were probably near equilibrium when the daily urine and fecal I^{131} reached a plateau. All the iodide pools within the body were considered in isotopic equilibrium with the diet when (a) the daily sum of urine and fecal I^{131} equaled the daily dose of I^{131} and (b) the specific activity of the diet equaled the specific activity of the thyroid gland (Van Middlesworth and Truemper, 1955). Determination of chemical iodine in the diet was subject to serious errors and assay of chemical iodine in the thyroid required sacrifice of the animal. Therefore, criterion (a) was the most useful index of equilibrium, although (a) may be practically satisfied with slowly exchangeable, unequilibrated pools.

The positive iodide balance with triiodothyronine feeding probably indicated that the release of thyroid hormone was stopped but the iodide trap continued to store iodide in the hyperplastic thyroids.

Propylthiouracil blocked iodide accumulation by the thyroid. This resulted in urinary excretion of dietary iodide together with the iodide from breakdown of hormone. Accordingly, the data would indicate at least 70% of the daily dose of iodide was reused each day in these goitrous rats.

So far as the author is aware, there is no previous publication of the method described here for establishing biological equilibrium with a relatively short-life isotope. Some of the applications of this tagged diet procedure are as follows:

(a) Iodide balance study.
(b) Determination of reused iodide.
(c) Measurement of the functional total iodine pool of thyroid (possibly by external counting).
(d) Measurement of functional body iodine pool.
(e) Determination of PBI and plasma iodide repeatedly on small volumes of plasma.
(f) Theoretically, the total iodide, triiodothyronine and other pools of the thyroid could be measured if this method were combined with separation methods such as chromatography.

SUMMARY

An isotopic method is described in which the iodide balance of rats was followed for 8 times the half-life of the isotope.

Rats maintained on a low iodide goiter producing diet excreted most of their iodide in the feces unless thyroid metabolism was disturbed.

The total functional iodine pool has been measured in goitrous rats. In these animals, the feeding of triiodothyronine resulted in positive iodine balance until 5 days after the triiodothyronine was discontinued.

Acknowledgment

The author wishes to express his appreciation to Mr. Alfred Intoccia and Mr. Joseph M. Valdez for their technical assistance in this investigation.

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ENDOCRINE FACTORS IN THE WATER METABOLISM OF THE DESERT MAMMAL, *G. GERBILLUS*[1]

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IN RECENT years attention has been called to some of the mechanisms which allow the survival of various species of mammals in the hot and waterless circumstances of the desert. In 1947–48, the Schmidt-Nielsens (K. Schmidt-Nielsen, and B. Schmidt-Nielsen, 1952) were able to work out a water balance for several species of mammals living in the desert of southeastern Arizona. In the laboratory, these animals were given no water *per se*. They derived water from the moisture content of their dry grain diet (about 5% water) and from its water of metabolism. On the deficit side, water loss occurred in the urine and by evaporation from the lungs. The loss from sweat and in the feces appeared to be negligible. Probably the most important aspect of their water account is their conservation of water by excreting a highly concentrated urine. Several species of desert mammals have been reported to produce specimens of urine with urea concentration of 3 to 4 M/L., and an electrolyte content equivalent to 1.2 to 1.5N NaCl (Howell and Gersh, 1935; K. Schmidt-Nielsen and B. Schmidt-Nielsen, 1952). These concentrations are of a different magnitude from those noted in other mammalian species, including man.

In the present study, the objectives were to observe the renal concentrating capability of the Egyptian desert mammal, *G. gerbillus*, under varying degrees of water deprivation and to learn to what extent its concentrating ability could be modified by adrenalectomy or by exogenous adrenal and posterior pituitary hormones.

METHOD AND MATERIALS

Gerbillus gerbillus gerbillus (Olivier 1801) is a small desert rodent that has a wide distribution across the North African Desert. The animals used in the present study were trapped by Bedouin hunters near the village of Abu Ruash, about 20 miles from the center of Cairo, Egypt. Animals were available in large numbers in summer and fall, but were obtainable in numbers too few for experimentation in winter and spring.

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[1] The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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Only adult male animals were used, weighing from 17 to 25 gm. The animal is depicted in Figure 1.

In his natural environment, the gerbillus lives in deep burrows. He is a nocturnal animal, foraging when the desert is cool and relatively humid. In the area of the Western Desert of Egypt where the animals studied were captured, succulent plants are sparse and the gerbillus' main sustenance is dry seed.

After capture, the animals were housed in glass jars provided with wood shavings. Except when noted otherwise, all observations were carried out on animals housed in a constant temperature and humidity chamber.[3] Temperature was maintained at 21.8°C.

FIG. 1. *G. gerbillus*, adult male.

and the relative humidity at 50%, conditions approximating those found in burrows in the local area (Hoogstraal and Briscoe, 1954).

Diets. At no time did any of the animals receive drinking water. During the course of the experiments, four diets were used: (a) pearled barley, (b) pearled barley plus freshly-cut carrots, (c) soy beans, and (d) salted pearled barley. Salted pearled barley was prepared by the method described by Schmidt-Nielsen, Schmidt-Nielsen and Schneiderman (1948). The resulting barley contained somewhat less than 10% salt. Dry soy bean contains about 40% protein. All the grains used were stored in shallow pans in the constant temperature and humidity chamber. The water content of the grains was determined periodically and was always found to be in the range of 9 to 11%.

Method of collecting urine. The major technical difficulty encountered in studying variations in renal function in the gerbillus was the collection of a sufficient volume of urine for chemical analysis. Even a relatively mild disturbance, such as handling or

[3] "Climatizer," manufactured by the American Instrument Company, Silver Spring, Maryland.

weighing the animal, would decrease the concentration of the urine subsequently formed. The most satisfactory method found was the collection of urine directly from the end of the penis immediately after spontaneous micturation. The animal was picked up gently from his cage, turned on his back and teased with forceps. Using this method, a suitable sample could be obtained from 50 to 70% of the animals so handled. Time of day was important; collections were most successful when made during the first two or three hours after sunset.

Pipettes. The constriction type pipette was used to collect the droplet of urine. They were made in the laboratory from soft glass tubing in a method similar to that described by Levy (1936). Each pipette was calibrated by two technicians, using an iodiometric titration method described by Balekjian and Burns (1955). The volumes obtained were within 2% of each other, and ranged from 3.5 to 14 microliters, After collection, the content of the pipette was introduced into a 2 ml. volume of distilled water.

Adrenalectomies. Adrenalectomies were performed under sodium pentobarbital anesthesia administered intraperitoneally. The dosage employed was 0.008 ml. per gram of body weight of a 750 mg. % solution. The adrenals were reached without difficulty through a posterior approach. The adrenal was encapsulated and was similar in appearance to the adrenal of the rat. No accessory glands were found. In sham operations, each adrenal was exposed but not removed.

Hormone preparations and administration. An aqueous suspension of cortisone[4] and an aqueous adrenal cortical extract[5] were used for adrenal hormone replacement. Two posterior pituitary preparations were used. One was an aqueous solution;[6] the other was vasopressin tannate suspended in oil.[7] Vasopressin tannate was given in an arbitrarily large dose, 0.25 units per animal, subcutaneously 24 hours before the collection of urine samples. In one experiment (No. 4, first urine collection), 2 doses were given, one 24 hours before the collection time, the second 12 hours later. Aqueous posterior pituitary extract, because of its rapid rate of absorption after subcutaneous administration, was injected 5 hours before urine collections were made.

Blood Samples; Organ Weights. Blood specimens and the adrenals and kidneys of the gerbillus were obtained at the time the animal was sacrificed. A volume of 0.2 to 0.3 ml. of blood could usually be obtained from the aorta by aspiration using a 27 needle. Organs were removed immediately. Extraneous tissue was dissected away and the organs weighed in a weighing flask on a Mettler (Switzerland) balance, accurate to 0.1 mg.

Chemical methods. Both urine and blood urea nitrogen were determined in duplicate by a modification (Seligson and Seligson, 1951) of the micro method of Conway and O'Malley (1942). The method used in determining total electrolyte concentration in urine and plasma is a modification of the one described by Holm-Jensen, (1947). In all data contained in this report, the total electrolyte concentration is reported in terms of equivalents of NaCl. When possible, a second urine sample was obtained and was used for the determination of the total nitrogen concentration by the micro-Kjeldahl method (Keys, 1940).

Statistical Analysis. When indicated, the standard error of the mean was calculated and intergroup comparisons made using student "*t*"; a P value of .05 or less was considered significant.

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[4] "Cortone" (Merek), containing 25 mg. cortisone per ml.

[5] "Cortigen" (Richter Company), containing 8 "corticodynamic units" per ml.

[6] "Infundibulin" (Evans Company, Liverpool, England), containing 10 pressor units per ml.

[7] "Pitressin Tannate in Oil" (Parke, Davis and Company, Limited, of London), containing 5 pressor units per ml.

RESULTS

I. Preliminary observations

Body weight, adrenal and kidney weight, concentration of urea and electrolytes in urine and blood urea nitrogen were measured in three groups of control animals (Experiment 1). The first group consisted of animals that were being maintained on barley and carrots; the second was made up of animals that had been captured within 24 hours of the time of sacrifice, and in the last were animals that had been on pearled

TABLE 1. EXPERIMENT 1. MEAN BODY, ADRENAL AND KIDNEY WEIGHT, URINE ELECTROLYTE AND UREA CONCENTRATION, AND BLOOD UREA NITROGEN, *G. gerbillus*

	Group I From reserve animals on carrots and barley	Group II Sacrificed within 24 hr. after capture	Group III Reserve animals on pearled barley only
Body weight, grams	21.1 (9)*	19.7 (11)	20.0 (8)
Urea in urine, M/L.	1.12 ± 0.34† (9)	1.32 ± 0.16 (11)	—
Total electrolytes in urine (as NaCl) Eq./L.	0.476 ± 0.055 (9)	0.617 ± 0.035‡ (11)	—
Blood urea nitrogen, mgm. %	54 ± 5 (4)	53 ± 7 (7)	57 ± 7 (8)
Kidney weight, mgm.	168 (8)	151 (8)	163 (8)
Adrenal weight, mgm.	10.4 (8)	7.2 (8)	9.4 (8)
K.W./B.W. $\times 10^3$	7.57 ± 0.27	7.72 ± 0.28	8.13 ± 0.24
A.W./B.W. $\times 10^4$	4.72 ± 0.41	3.69 ± 0.21§	4.78 ± 0.39

* Number in parenthesis is number of observations.
† Standard error.
‡ Significantly different from Group I ($p < 05$).
§ Significantly different from Group I and III ($p < 05$, $p < 02$ respectively).

barley alone for a week or more. None of these animals had been kept in the constant temperature chamber. The data collected are presented in Table 1. When kidney and adrenal weights were expressed as a ratio to the body weight, and the mean value of each group compared statistically, no difference was found in the kidney to body weight ratio. However, the adrenal to body weight ratio of the freshly captured group, Group II, was significantly less than that of the other two groups, suggesting that the gerbillus found the laboratory environment more stressful than that of the desert.

II. *Effect of Diet on Concentration of Electrolytes and Urea*

Of the four diets used, soy bean and salted barley could be considered "challenging diets," requiring conservation of water beyond the capability

TABLE 2. EXPERIMENT 2. WEIGHT LOSS AND MORTALITY ON VARIOUS DIETS, *G. gerbillus*

Day	0	1	2	3	4	% wt. loss	% mortality
Pearled barley	19.1 (8)*	17.8 (8)	17.3 (8)	16.5 (8)	16.0 (8)	16	0
Salted barley	19.8 (12)	17.8 (12)	15.9 (8)	14.0 (5)	12.5 (2)	37	83
Soy bean	17.4 (12)	15.9 (12)	15.0 (10)	14.1 (8)	14.0 (8)	20	33

* Number of animals.

of the animal. In Experiment 2, the two "challenging diets" were compared with the standard diet of pearled barley in the following manner: thirty-two recently-acquired animals were divided into three groups, and given salted barley, pearled barley, and soy bean respectively. Urine collections were made on Days 1, 2 and 3 of the diets. Weight and mortality figures are contained in Table 2, and urine urea and electrolyte figures in Tables 3 and 4, respectively.

The animals used in the above experiment had been captured just a day before the experiment started. Experiment No. 3 was designed to show the effect of the preceding dietary experience on the animal's ability to cope with the experimental regimen. Groups III and IV were made up of animals that had had a two week period on the barley-fresh carrot diet. In Groups I and II, animals were used that had been maintained on pearled

TABLE 3. EXPERIMENT 2. EFFECT OF DIET ON CONCENTRATION OF URINE ELECTROLYTES, *G. gerbillus*

Diet	Day 1	Day 2	Day 3
Pearled barley	0.834 Eq./L.	0.546	0.388
	0.684	0.929	0.505
	0.514	0.456	0.257
	1.119	0.760	
	0.534	0.407	
	Mean = 0.737	0.620	0.383
Salted barley	1.065	0.833	
	1.170	1.362	
	1.067		
	0.503		
	0.945		
	0.719		
	Mean = 0.912	1.098	
Soy bean		.858	
	0.542	.747	0.842
	1.047	1.061	0.710
	0.729	0.536	0.413
	1.060	0.955	0.708
	1.280	0.837	0.713
	Mean = 0.932	0.832	0.617

TABLE 4. EXPERIMENT 2. EFFECT OF DIET ON UREA CONCENTRATION, *G. gerbillus*

Diet	Day 1	Day 2	Day 3
Pearled barley	2.32 M/L.	1.83	1.02
	1.32	2.66	1.88
	1.58	1.57	0.84
	1.55	2.68	
		1.11	
	Mean = 1.87	1.97	1.25
Salted barley	1.43	0.61	
	0.75	1.32	
	1.10		
	0.99		
	1.62		
	2.54		
	Mean = 1.41	0.97	
Soy bean	1.01	2.59	1.80
	2.16	3.13	1.82
	2.61	2.01	2.13
	2.47	3.04	1.74
	2.32	3.41	2.56
	Mean = 2.11	2.84	2.01

barley alone. Group I was then put on barley plus carrots; Group II, barley alone; Group III, barley alone; and Group IV, salted barley. Weights were obtained daily. At the end of 18 to 37 days, the animals were sacrificed and blood samples obtained. Table 5 contains the results.

The initial weight in Groups III and IV was about 4 gm. greater than in Groups I and II. Group IV, fed salted barley, had a mortality of 44% and a mean weight loss of 38% over an 18 day period. In the foregoing experiment (see Table 2) salted barley fed animals had an 83% mortality and a 37% weight loss. A period of increased water intake thus seemed to enhance the animals' ability to survive a subsequent period on a dehydrating diet. When the blood urea nitrogen concentrations of Groups II, III and IV were compared with those of Group I, each was significantly higher

TABLE 5. EXPERIMENT 3. OBSERVATIONS ON CHANGES IN BODY WEIGHT, MORTALITY AND BLOOD CHEMISTRY IN *G. gerbillus* UNDER VARIOUS DIETARY REGIMENS

	Group I (16)*	Group II (8)	Group III (16)	Group IV (16)
Diet before period of observation	pearled barley	pearled barley	carrots and barley	carrots and barley
Diet during period of observation	carrots and pearled barley	pearled barley	pearled barley	salted barley
Period of observation, days	31	37	18	18
Body weight, gm., beginning of observation period	18.4	19.1	24.0	23.8
Body weight, gm., end of observation	19.4	20.7	18.9	14.8
% change in body weight	+5%	+8%	−21%	−38%
Mortality %	13%	25%	0%	44%
Blood urea mgm./100 ml.	27.3±4.75 (11)	48.7±6.36 (6)	62.1±7.69 (13)	80.6±14.15 (8)
p (against Group I)		.02	.01	.01
Plasma total Electrolytes, mEq./L.	149±1.38 (9)	—	—	—

* Number of animals or observations.

than Group I. The highest figure was noted in Group IV, 81 mg.%. Plasma total electrolytes could be measured only in Group I and was 149 mEq./L.

III. *Observations on the Effect of Antidiuretic Hormone on the Concentration of Urea and Total Electrolytes*

Experiment No. 4 was devised to test the influence of exogenous antidiuretic hormone at a time when the animal's own posterior pituitary would be relatively inactive, i.e., during a time of ample water content in the diet. In it, 32 animals were placed on carrots plus barley. Eight additional animals were on barley alone. After one day on this regimen, 16 of the animals receiving carrots were injected with vasopressin tannate in oil. All

TABLE 6. EXPERIMENT NOS. 4 AND 5. EFFECT OF VASOPRESSIN TANNATE AND DIET ON THE CONCENTRATION OF UREA AND TOTAL ELECTROLYTES IN THE URINE, *G. gerbillus*

		Group I Carrots and barley		Group II Carrots and barley and vasopressin	Group III Barley alone	Group IV Barley alone +vaso-pressin§	Group V Salted barley
First collection*	Urea M/L.	1.33 ±0.24 (7)	p 1	1.75 (0.15) (13)	1.83 ±0.24 (7)	1.81 ±0.14 (13	1.24 ±0.16 (11)
						p <02	
	Total Electrolytes Eq./L. (as NaCl)	0.557±0.031 (7)	5	0.587±0.045 (13)	0.711±0.125 (7)	0.457±0.044 (13)	1.297±0.091 (11)
						p <01	
Second collection†	Urea	0.92 ±0.11 (7)	1	1.42 ±0.20 (15)	—	1.30 ±0.191 (7)	0.77 ±0.194 (3)
						p <1	
	Total Electrolytes	0.515±0.113	05	0.744±0.061	—	0.517±0.052 (7)	0.722±0.123
						p <1	
Third collection‡	Urea	—		—	—	1.40 ±0.166 (7)	1.08 ±0.525
						p <05	
	Total Electrolytes	—		—	—	0.564±0.034 (7)	0.714±0.032 (3)
						p <05	

* Day 2 of period of observation for groups I, II, and III. Day 5 for Groups IV and V.
† Day 6 of observation for Groups I and II; Day 13 for Groups IV, V.
‡ Day 15 for Groups IV, V.
§ Given before first collection only.

other animals were sham-injected. Twenty-four hours later, urine samples were collected from all three groups. Six days later, the injection of vasopressin was repeated in the same animals as in the first experiment, and again urine collections were made 24 hours later. The results are shown in Table 6 (Groups I, II, III). The animals that received carrots and vasopressin showed statistically no greater concentration of urea or total electrolytes than those that did not receive vasopressin, except that electrolytes were significantly more concentrated in the treated group in the second collection.

In Experiment No. 5, concentrations of urea and total electrolytes of a salted barley fed group were compared with those of a group fed barley, and that, in addition, received vasopressin tannate in oil 24 and 12 hours before the first urine collection. The animals had been on their respective diets for 5 days when the first urine collection was made. Second and third collections were made on Days 11 and 13 of the diet regimen but no vasopressin was given antecedent to these collections. The results of the

analyses are shown in Table 6 (Groups IV and V). The concentration of electrolytes achieved by the animals on salted barley was extremely high. The mean value of 11 animals was 1.3N (7.6%). The highest individual concentration noted was 1.6N (9.4%). The mean urea concentration of the animals on pearled barley and injected with vasopressin was 1.8 M/L., the same figure obtained in earlier experiments (see Table 4; Table 6, Group III). The mean electrolyte concentration of this group was 0.457N, somewhat lower than that noted in animals on the same diet studied earlier.

In one experiment the aqueous posterior pituitary extract was used. A total of 18 animals were divided into three groups. The first group was injected with 0.1 ml. of sterile water; the second was injected with 0.1 ml. of aqueous posterior pituitary extract (0.01 unit); the third received 0.05 ml. (0.005 unit). All animals had been on a pearled barley diet for a minimum of four days. At neither dose level did the aqueous posterior pituitary enhance the concentration of the total electrolytes or urea.

IV. *The Effect of Adrenalectomy and Hormone Replacement on Survival and Concentration of Urinary Urea and Total Electrolytes.*

In a series of preliminary experiments performed in 1953, a large number of animals were subjected to adrenalectomy or sham operation. At a dose level of 5 mg. per day, replacement with cortisone appeared to enhance survival of adrenalectomized animals on a challenge diet (see Table 7).

To evaluate the effect on adrenalectomy and hormone replacement on the concentration of urinary urea and total electrolytes, a total of 80 animals were divided into four groups: Group I was adrenalectomized and received cortisone replacement, 5 mg. per day subcutaneously; Group II was sham operated. Group III was adrenalectomized and received no replacement therapy; and Group IV was adrenalectomized and received daily 4 units of aqueous extract of the adrenal cortex, subcutaneously. Ani-

TABLE 7. CUMULATIVE MORTALITY (%)—*G. gerbillus* MAINTAINED WITHOUT WATER

Series	Groups*	Day													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
#3	A (6)	0	0	17	67	83	83	100	—	—	—	—	—	—	—
Stock	B (5)	0	0	20	20	40	100	—	—	—	—	—	—	—	—
barley	C (5)	20	20	20	20	20	40	40	60	60	60	60	60	60	60
#4	A (10)	70	80	40	100	—	—	—	—	—	—	—	—	—	—
Salted	B (9)	45	56	56	89	89	100	—	—	—	—	—	—	—	—
barley	C (10)	40	40	50	60	60	70	70	70	80	80	80	80	80	80
#5	A (10)	10	60	70	80	100	—	—	—	—	—	—	—	—	—
Salted	B (11)	27	36	45	45	55	55	73	73	82	82	82	82	82	82
barley	C (18)	25	25	25	25	38	38	38	38	38	38	63	63	63	65

* In all series:
Group A = Adrenex without replacement.
Group B = Adrenex with 2.5 mgm. cortone replacement after every day (in series 5, 5 mgm. per day).
Group C = Sham-Operated Animals.

mals in Groups II and III were sham-injected daily. On days in which urine specimens were collected, the animals were injected before 8:30 a.m., so that approximately 12 hours elapsed before collections were made.

On the day of operation and for the next two days all animals received fresh carrots as well as pearled barley, and all animals were given 0.5 ml. of 0.85% saline. On Day 4, the last injection of physiological saline solution was given, and the diet was changed to one of pearled barley alone. On Day 6 the diet was changed to salted barley. Thus, in three stages, all

TABLE 8. CUMULATIVE MORTALITY AND DAILY WEIGHT IN ADRENALECTOMIZED AND SHAM-OPERATED *G. gerbillus*, WITH AND WITHOUT REPLACEMENT TREATMENT

	Day	0	3	5	8	10	% wt. loss day 10
Group I	Wt.						
Adrenex+cortisone*	(grams)	23	22	20	18	15	35
Group II							
Sham-operated	(grams)	24	23	23	20	18	25
Group III							
Adrenex—no replacement	(grams)	25	23	23	21	19	24
Group IV							
Adrenex+ACE†	(grams)	26	24	22	20	18	31
	Mort.						
Group I	(%)	—	10	20	55	70	
Group II	(%)	—	5	5	10	10	
Group III	(%)	—	25	71	83	83	
Group IV	(%)	—	44	44	44	50	

* 5 mg. per day.
† 4 units per day.

animals were shifted from a regimen containing ample water to one severely restricted in water. Urine collections were made in the evenings of Days 5 and 8, after the animals had been on the intermediate diet 36 hours and the severe one 60 hours, respectively.

Table 8 tabulates the course of events in all four groups with regard to change in weight and mortality throughout the period of observation. By Day 10, weight loss was marked in all groups, ranging from 24% in Group III to 35% in Group I. Mortality by Day 10 was high (50 to 83%) in all adrenalectomized groups and low (10%) in the sham-operated group.

Table 9 summarizes the concentration of urea and total electrolytes in the urines collected. The most striking finding in the first urine collection was the failure of concentration of urea in all animals that were adrenalectomized. The sham-operated group demonstrated moderate concentration, statistically significantly higher than in any group adrenalectomized, except Group III. In the second collection, the urea concentration of Group II was statistically greater than any other group. The administration of cortisone or adrenal extract did not enhance the concentration of urea.

The figures for the total electrolytes were quite different. In the first collections, all animals, regardless of operation or treatment, were found to have total electrolyte concentrations within a narrow range, from 0.503N to 0.616N. In the second urine collection, while on salted barley, all groups except IV showed an increase in concentration of electrolytes; in Groups I and II, the increase was statistically significant. There were more variations in concentration among the groups in the second urine collection than the first, but no statistically significant differences between any two groups were found.

TABLE 9. URINE CONCENTRATION OF UREA AND TOTAL ELECTROLYTES IN ADRENALECTOMIZED AND SHAM-OPERATED *G. gerbillus*

	Urea M/L.		Total electrolytes N. as NaCl	
	Collection #1*	Collection #2†	Collection #1	Collection #2
Group I Adrenex+cortisone, 5 mg/d.	0.59±0.06 (17)	0.62±0.12 (8)	0.503±0.030 (17)	0.782±0.125 (8)
Group II Sham	1.27±0.08 (19)	1.10±0.09 (13)	0.513±0.035 (19)	0.753±0.030 (13)
Group III Adrenex—no replacement	0.70±0.11 (10)	0.57±0.13 (5)	0.576±0.051 (10)	0.622±0.116 (5)
Group IV Adrenex+adrenal extract, 4 u./d.	0.57±0.06 (8)	0.36±0.04 (8)	0.616±0.081 (8)	0.610±0.060 (8)
p Values:				
Groups II vs. I	p<01	<01	>5	>5
Groups II vs. III	p>1	<01	>3	>5
Groups II vs. IV	p<01	<01	>05	>05
Groups III vs. IV	p>3	>05	>5	>5
Groups I vs. IV	—	—	>1	>2

* Day 5, animals on pearled barley.
† Day 8, animals on salted barley.

DISCUSSION

There seemed no doubt that the species of desert mammal under study, *G. gerbillus*, was capable of concentrating urea and electrolytes to a marked degree. Under the stimulus of "challenging" diets, the greatest individual figures were 3.41 M/L. (21%) urea and 1.6N (9.4% as NaCl) total electrolytes. These are as high or higher than comparable figures reported for other species of desert mammals.

The manner in which these several species achieve these marked concentrations of urinary excretory products has received some attention. Howell and Gersh (1935), studying the kangaroo rat, produced evidence suggesting that reabsorption of water occurred not only in the renal tubules but also in the collecting ducts of the papillae and from the bladder itself. Schmidt-Nielsen *et al.* (1952), however, injected saline and urea solutions into the ligated bladder of the mammal *Dipodomys deserti* and failed to note change in either the volume or concentration of any solution used. In a series of clearance studies, Schmidt-Nielsen (1951) demonstrated that the glomerular filtration rate of *D. merriami* and *D. spectabilis* were

comparable to the white rat. However, in the dry fed desert mammal, the reabsorption of water was very marked, the urine being more concentrated than the plasma by a factor of 1000. Comparing urea and inulin clearances, Schmidt-Nielsen concluded that the desert mammal must actually secrete urea into the renal tubules. In this respect, the desert mammal would appear to be unique, since this phenomenon has been observed in no other mammal.

The possibility that the desert mammal's unusual tubular concentrating ability may be dependent on extrarenal influences, such as hormonal ones, has not been thoroughly explored. Ames and Van Dyke (1950) found that the pituitary of *D. merriami* contained more antidiuretic hormone (0.9 milliunits/μg.) than did the pituitary of the laboratory white rat (0.3 milliunits/μg.). Similarly, the concentration of antidiuretic hormone excreted in the urine of the desert mammal studied was much higher than that found in urine from the laboratory rat or dog.

Nichols (1949), placed three groups of kangaroo rats on a dry diet of barley, on a diet high in sodium, and on one high in potassium, respectively. Animals maintained on the latter two diets eventually died of dehydration. None of the animals showed evidence of lipid depletion of the adrenals when autopsied. White rats maintained on similar regimens showed marked lipid depletion of the zona fasciculata and zona reticularis.

Under the conditions of the experiments employed, antidiuretic hormone caused no apparent augmentation of concentration of the urinary constituents measured. If ADH normally plays an important role in enhancing the concentration of urinary solutes, one might have expected more response from the exogenously-administered hormone. However, the dose given might have been poorly absorbed or there may be a species difference in the biological activity of commercially available ADH and that of the gerbillus. It was not surprising that a large dose of cortisone improved the survival of adrenalectomized animals on a "challenge" diet. This might occur whether or not the hormone had any specific effect on the renal tubular function. No adrenalectomized animal, regardless of replacement therapy, could produce urine with the expected concentration of urea. On the other hand, as far as electrolytes are concerned, sham-operated and adrenalectomized animals put out respectable concentrations and were capable of putting out a still more concentrated urine when salt was added to the diet.

Thus, no evidence was adduced to indicate that exogenous hormones can influence urine solute concentration in the gerbillus. If posterior pituitary or adrenal hormones are important factors in supporting the tubular work required to achieve the impressive concentrations of urea and electrolytes, then more direct methods will be required to demonstrate this.

SUMMARY

G. gerbillus, a small desert mammal of the Egyptian Desert, was observed in the laboratory to survive indefinitely on a diet of dry barley.

On dry "challenge" diets that imposed an excretory load of nitrogen or electrolytes, the gerbillus put out markedly concentrated urine; the highest concentration of urea noted was 3.41 M/L. (21%), and that of total electrolytes was 1.6N (9.1%).

Vasopressin tannate suspended in oil and aqueous posterior pituitary extract both failed to enhance urine concentration of urea or total electrolytes.

Replacement therapy with cortisone improved survival of adrenalectomized animals.

Adrenalectomized animals with cortisone or adrenal extract replacement or with no replacement were able to maintain urine concentration of electrolytes as well as sham-operated controls.

Sham-operated controls excreted significantly higher concentrations of urea than did adrenalectomized animals, with or without replacement treatment.

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REGULATION OF THE SECRETION OF ALDOSTERONE-LIKE MATERIAL[1]

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BY MEANS of paper chromatography and bioassay, a highly active sodium-retaining material was demonstrated in the effluents from isolated calf adrenals perfused with an artificial medium (Ungar *et al.*, 1954). Although isolation and identification has not been achieved, it is highly probable that this potent electrolyte regulating substance is actually aldosterone. Preliminary experiments indicated that the calf adrenal secreted approximately 0.3 μg. of aldosterone equivalent per gram of tissue per hour. In the present studies, the perfusion medium was modified by the addition of ACTH or growth hormone, and by alterations in the sodium and potassium concentrations in order to investigate the influence of these factors on the production and release of sodium-retaining material.

MATERIALS AND METHODS

Control groups of 4 glands consisting of 2 left and 2 right adrenals were perfused for 2 hours at 37.5°C. in a multicycle system with a liter of a synthetic fluid. The composition of this perfusion medium and the perfusion technique have already been detailed elsewhere (Rosenfeld, 1955). The experimental groups comprising the 4 contralateral organs were similarly and simultaneously perfused in another apparatus with a modified artificial medium. The modifications in two studies involved the addition of either 10–25 units/L./hr. of ACTH (Upjohn's Corticotrophin) or 125 mg. of growth hormone[3] (Armour's Somatotropin). In the third study, the sodium and potassium concentrations of the experimental perfusion medium were changed from 149 and 3.5 to 125 and 25 m.eq./L., respectively. The difference in weight between each experimental and contralateral con-

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[3] Generously supplied by Dr. S. L. Steelman, Biochemical Research Dept., The Armour Laboratories.

trol group of glands was less than 5%. At the termination of each perfusion, the effluents were frozen in a dry ice-acetone mixture and kept frozen until extracted for corticoids.

Each thawed perfusate was extracted 4 times with fresh portions of one-third volumes of redistilled ethyl acetate. The ethyl acetate extract was then treated with sodium sulfate and evaporated to dryness under vacuum. This residue was chromatographed on paper for 72 hours in a toluene-propylene glycol system (Zaffaroni *et al.*, 1950). The zone migrating in front of cortisol which would contain a mixture of cortisone and aldosterone was eluted and prepared for bioassay in the growth hormone and Na/K studies. In two experiments with ACTH, this eluted zone was rechromatographed in a benzene-ethyl acetate-methanol-water system (Bush, 1952) in order to separate aldosterone from cortisone.

The sodium-retaining activity of the eluted aldosterone zones was determined by bioassay. The method, a modification of the one by Marcus *et al.* (1952), employs the bilaterally-adrenalectomized male rat weighing between 135 and 150 gm. Each animal was injected intraperitoneally with 45 mg. of sodium chloride and the urine was collected for 6 hours. The urinary sodium was determined by flame photometry using a Perkin and Elmer Model XX instrument and an internal standard of lithium. The experimental and control zones were usually assayed at more than one concentration and concurrently with a solvent control group (20% ethanol) and two other groups which received desoxycorticosterone at 6 and 25 μg. dosage levels. In those instances where a single concentration was chosen, duplicate bioassays were performed. The 8 animals employed in the bioassay of each unknown, solvent control, or standard, were grouped in pairs. Differences in sodium excretion between experimental and control perfusions were evaluated statistically where possible but it was not deemed advisable to translate these results in terms of aldosterone equivalents.

RESULTS

The detailed results concerning the influence of ACTH on the secretion of aldosterone by the perfused calf adrenal are summarized in Table 1.

TABLE 1. INFLUENCE OF ACTH ON THE SODIUM RETAINING ACTIVITY OF CALF ADRENAL PERFUSATES
(8 adrenalectomized rats per group)

Exp. No.	Zone* analyzed	Aliquot per animal	Conc. of ACTH U./L.	Excretion of sodium in mg. per 100 gm. per hour (range)				
				Solvent control	Desoxycorticosterone standard		Control perfusate	Experimental perfusate
					6 μg.	25 μg.		
1	A	1/20	10	1.31 (0.99–1.67)	1.63 (1.22–1.96)	0.88 (0.41–1.14)	1.29 (1.02–1.65)	0.93 (0.62–1.19)
	A	1/20	10	1.88 (1.09–2.65)	1.76 (1.52–2.06)	1.52 (1.44–1.71)	1.25 (0.76–1.65)	1.19 (0.63–1.58)
2	A	1/15	20	1.99 (1.69–2.49)	1.25 (0.93–1.57)	0.90 (0.82–0.96)	1.65 (1.14–2.05)	0.84 (0.73–0.95)
3	E	1/60	10	1.72 (1.38–2.19)	1.59 (1.06–2.03)	0.93 (0.50–1.15)	0.97 (0.87–1.11)	1.12 (0.59–1.56)
	E	1/60	10	1.40 (0.97–1.79)	1.04 (0.86–1.34)	0.90 (0.51–1.12)	0.94 (0.82–1.00)	1.36 (0.83–1.6)
	E	1/60	10	1.63 (1.12–2.05)	0.84 (0.82–0.92)	0.67 (0.53–0.82)	1.47 (1.0–2.07)	1.30 (1.0 –1.54)
4	E	1/30	25	2.16 (1.20–2.06)	1.41 (0.7 –2.37)	0.77 (0.39–0.91)	1.59 (1.20–2.06)	1.27 (0.74–1.81)
	E	1/15	25	1.40 (0.97–1.79)	1.04 (0.86–1.34)	0.90 (0.51–1.12)	0.96 (0.79–1.15)	0.90 (0.55–1.29)

* The zones analyzed were either (A) aldosterone, essentially free of cortisone, or (E) aldosterone containing cortisone. The amount of cortisone in the latter zones was not enough to interfere with the bioassay.

It will be noted that in some experiments the zone containing aldosterone plus cortisone (E) was employed for assay. The quantity of cortisone present in the latter was not enough to interfere with the sodium-retaining assay.

The initial bioassay data of the first ACTH experiment indicated a tendency toward increased sodium-retaining activity when each animal received 1/20th of the total extract; however, essentially no difference was observed when the bioassay was repeated with a similar aliquot. The pooled data of these two bioassays show a mean sodium excretion for the control perfusate of 1.27 ± 0.11 as compared to one of 1.06 ± 0.12 mg./100 gm./hr. for the perfusate obtained from ACTH-stimulated adrenals. When these data were analyzed statistically, a t value of 1.273 was found indicating that the difference between the control and experimental perfusions was not statistically significant. In the second experiment in which 1/15th of the total extracts was administered, a striking difference was noted. The sodium excretion of animals receiving the control perfusate extract was 1.65 as compared to 0.84 mg./100 gm./hr. for the animals injected with the ACTH perfusate extract. The data of the third and fourth

TABLE 2. INFLUENCE OF GROWTH HORMONE* ON THE SODIUM RETAINING ACTIVITY OF CALF ADRENAL PERFUSATES
(8 adrenalectomized rats per group)

Exp. No.	Aliquot per animal	Excretion of sodium in mg. per 100 gm. per hour (range)				
		Solvent control	Desoxycorticosterone standard		Control perfusate	Experimental perfusate
			6 μg.	25 μg.		
1	1/20	2.52 (2.04–2.92)	1.30 (1.05–1.61)	1.09 (0.89–1.31)	1.49 (1.09–1.86)	1.28 (1.05–1.62)
	1/80	1.18 (1.06–1.38)	1.28 (1.03–1.61)	0.74 (0.39–1.39)	1.35 (0.59–1.95)	0.79 (0.76–0.83)
2	1/20	1.18 (1.06–1.38)	1.28 (1.03–1.61)	0.74 (0.39–1.39)	0.98 (0.64–1.24)	1.00 (0.86–1.06)
	1/80	1.72 (1.38–2.19)	1.59 (1.06–2.03)	0.93 (0.50–1.15)	1.59 (1.33–1.92)	1.51 (1.36–1.60)
3	1/20	2.12 (1.83–2.42)	1.68 (1.18–2.43)	0.94 (0.86–1.0)	0.65 (0.4–0.8)	0.89 (0.75–1.12)
	1/80	1.80 (1.60–1.96)	1.28 (1.17–1.53)	1.02 (0.97–1.13)	1.32 (0.75–2.17)	1.74 (1.10–2.60)
4	1/20	1.72 (1.38–2.19)	1.59 (1.06–2.03)	0.93 (0.50–1.15)	1.21 (1.06–1.55)	1.27 (0.92–1.54)
	1/80	1.40 (0.97–1.79)	1.04 (0.86–1.34)	0.90 (0.51–1.12)	1.21 (0.87–1.52)	1.05 (0.94–1.21)
	1/80	1.63 (1.12–2.05)	0.84 (0.82–0.92)	0.67 (0.53–0.82)	1.52 (0.89–2.14)	2.06 (1.45–2.56)

* 125 mg./l. of Armour's Somatotrophin.

experiments showed no essential difference in the Na-retaining activity of the effluents of unstimulated and ACTH-stimulated glands.

Four experiments were conducted to ascertain the influence of growth hormone on the secretion of aldosterone-like material. The results, summarized in Table 2, indicate that the growth hormone exerted no significant action. Experiment 1 showed some tendency toward an increased output of aldosterone-like material in the experimental perfusate but the

TABLE 3. INFLUENCE OF Na/K IMBALANCE* ON THE SODIUM-RETAINING ACTIVITY OF CALF ADRENAL PERFUSATES
(8 adrenalectomized rats per group)

Exp. No.	Aliquot per animal	Excretion of sodium in mg. per 100 mg. per hour (range)				
		Solvent control	Desoxycorticosterone standard		Control perfusate	Experimental perfusate
			6 μg.	25 μg.		
1	1/10	1.45 (1.27–1.78)	1.64 (1.53–1.95)	0.98 (0.74–1.37)	0.63 (0.34–1.10)	0.78 (0.45–1.31)
2	1/30	2.16 (1.40–2.80)	1.32 (1.22–1.45)	1.74 (1.29–2.44)	2.27 (1.85–2.54)	1.23 (1.04–1.31)
	1/15	1.91 (1.20–2.29)	1.21 (0.89–1.51)	1.01 (0.96–1.30)	2.19 (1.88–2.45)	1.05 (0.83–1.24)
3	1/30	1.51 (1.11–2.09)	1.26 (0.89–1.81)	0.52 (0.38–0.71)	0.78 (0.50–1.25)	0.84 (0.53–1.05)
	1/60	1.66 (1.02–2.31)	1.00 (0.58–1.65)	0.70 (0.64–0.77)	1.45 (1.10–1.81)	1.28 (0.99–1.65)
4	1/30	1.99 (1.91–2.04)	1.42 (1.09–1.60)	0.70 (0.47–0.94)	1.49 (1.12–1.97)	1.22 (0.87–1.52)
	1/60	2.12 (1.83–2.42)	1.68 (1.18–2.43)	0.94 (0.86–1.00)	1.54 (1.43–1.75)	1.24 (1.14–1.46)

* The Na and K concentrations of the experimental perfusion medium were 125 and 25 m.eq./l., respectively.

differences observed were certainly not significant. Experiments 2 and 3 were likewise negative. In Experiment 4, a small increased excretion of sodium was noted in one assay at the 1/80th of total extract level which was not statistically significant and was not observed in a repeat assay.

The influence of changing the Na/K ratio of the perfusing medium from 42/1 to 5/1 on the secretion of sodium-retaining material can be seen in Table 3. In the first of the four perfusion experiments performed a significant difference was not observed probably because the aliquot injection (1/10th) was too large and yielded a high sodium retention in the case of the control perfusate. At a level of 1/30th of the total perfusate materials (Exp. 2), the sodium excretion of the control perfusate was 2.27 mg./100 gm./hr. as compared to 2.16 for the solvent-treated animals. However, the material derived from the experimental perfusate gave a

significantly different sodium excretion value of 1.23 which was highly significant. The same striking difference in the Na-retaining activities of the experimental and control perfusates was also observed when they were bioassayed at a 1/15th dosage level. The sodium excretion values were 1.05 mg./100 gm./hr. for the experimental extract, 2.19 for the control extract, and 1.91 for the solvent control. The combined data from experiment 2, when treated statistically, gave a t value of 10.0 and a P value of less than 0.001 for the difference in sodium excretion produced by the control and experimental perfusate material.

In Experiment 3, 1/30th of the total control perfusate extract produced a high sodium retention while 1/60th yielded a sodium excretion value which did not differ from that of the solvent controls. At this lower level the experimental perfusate value of 1.28 mg./100 gm./hr. was not significantly different from the control perfusate value of 1.45. However, in Experiment 4, the Na/K imbalance again acted as a stimulus for the secretion of aldosterone-like material by the isolated calf adrenal. The pooled data for the 1/30th and 1/60th dosage levels showed a statistically significant decrease in sodium excretion for the experimental perfusate extract (1.23 ± 0.08 mg./100 gm./hr.) when compared to the control perfusate extract (1.52 ± 0.09).

DISCUSSION

At the present time, the control of aldosterone production and release is not well understood. Recent reports have suggested that pituitary hormones and changes in electrolyte concentrations in the blood stream may be important factors in the regulation of its biosynthesis and/or secretion. Studies of Venning and co-workers (1954) in humans indicated that ACTH did not modify the urinary excretion of aldosterone, whereas treatment with growth hormone appeared to increase the excretion of urinary aldosterone. These results are not consistent with other findings. Singer and Stack-Dunne (1954) have recently reported a reduced concentration of aldosterone in the adrenal venous blood of hypophysectomized rats. Treatment of these animals with ACTH partly restored the aldosterone content of the adrenal venous blood. Farrell and co-workers (1954), using hypophysectomized dogs, observed a distinct lowering of the glycogenic steroids in the adrenal venous blood, whereas the aldosterone level was only modestly affected. Treatment of the hypophysectomized dogs with ACTH induced no significant increase in aldosterone output. In our studies with the perfused calf adrenal isolated from the metabolic influences of other tissues, a level of 125 mg./L. of growth hormone produced no significant effect. The influence of ACTH on the isolated adrenal was suggestive of some modest increases in aldosterone secretion, in contrast to a two to threefold increase in 17-hydroxycorticosterone and corticosterone production. Thus, our findings appear to be more closely related to those of

Singer and Stack-Dunne than to those of Venning and Farrell. Since the growth hormone preparations employed are probably not pure substances, one might expect that their effect on the elaboration and release of Na-retaining material might vary. The variability in the effect of ACTH may also be due to differences in the preparations utilized. It would be desirable to repeat these experiments with some of the pure ACTH polypeptides that have recently been described by the Armour and Lederle laboratories.

Axelrad and co-workers (1954) have indicated that a low sodium intake in the human may act as a stimulus to the secretion of aldosterone as evidenced by the increased urinary output. In our studies of the influence of circulating electrolyte levels, the control Na/K ratio of 42 resembles the normal proportion of these plasma ions as opposed to the experimetal ratio of 5, which is abnormally low and simulates the deficiency of Na and excess of K that is obtained in adrenalectomy or adrenocortical insufficiency. Two bioassays were inconclusive since relatively high amounts of activity were observed in both the control and the experimental perfusates. In the two others, highly significant differences in Na-retaining activity were demonstrated. No significant change in 17-hydroxycorticosterone and corticosterone production could be detected in these perfusions. It is intended to refine this study by altering the Na and K concentrations separately in order to determine whether it is the low Na, high K, or both which serve as the direct stimulus for the release of aldosterone-like material by the isolated adrenal.

SUMMARY

The regulation of the secretion of aldosterone-like material by isolated calf adrenals was studied by means of perfusion technique involving an artificial medium. It was found that the addition of growth hormone did not exert a significant influence on the Na-retaining activity of the effluents. The stimulative effect of added ACTH was suggestive but not unequivocally demonstrated. A substantial Na/K imbalance in the artificial perfusion medium, produced by lowering the Na and increasing the K content, induced a significantly augmented output of aldosterone-like material.

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STEROID HORMONES AND THE DEVELOPMENT OF THE REPRODUCTIVE SYSTEM IN THE PULLET[1]

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THE reproductive system of the female bird undergoes remarkable changes at sexual maturity or when seasonal breeding occurs at which times weight and size increments of several thousand per cent occur in both the ovary and oviduct in only a few weeks or in only a few days. The extensive literature on the morphological aspects of these changes in birds has been reviewed (Breneman, 1955), but relatively little is known about the hormonal factors which influence the structural alterations. Fraps, Hooker, and Forbes (1948, 1949) determined that progesterone or a progesterone-like substance, is present in the fowl at about the time of ovulation. Kornfeld and Nalbandov (1954) demonstrated the presence of an estrogen in pullets at 16–20 days post-hatching and Romanoff (1949) reported that estrogen is secreted by the ovary of mature birds and that an estrogen can be found in the yolk of the egg. The presence of androgens in fowls is known only indirectly. Marked comb growth in cockerels and in pullets, however, is produced only by androgens (Breneman, 1938, 1942, 1955).

It is possible to measure simultaneously in female birds the effects of androgens and estrogens and since it seems probable that progesterone also is present, there is an opportunity to study in pullets the influence of three steroids on the anterior pituitary and ovary of the bird. This paper reports an investigation of steroid hormone action in pullets in an attempt to answer several specific questions: first, do estradiol dipropionate and testosterone propionate administered alone and in combination promote comb growth; second, does androgen inhibit or augment the effectiveness of estrogen in stimulating oviduct growth; third, do relatively large doses of steroid hormones inhibit the growth of the ovary and of the anterior pituitary; fourth, is the secretion of gonadotropin by the anterior pituitary inhibited by the sexogens?

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MATERIALS AND METHODS

Single comb White Leghorn chicks were used. The birds were kept in battery brooders and were apportioned into groups with uniform comb sizes and body weights at the beginning of each experiment. Anterior pituitary glands were removed quickly at autopsy, weighed individually, and stored in acetone for later administration to assay animals. The weights of the organs, including the small combs, were taken on a torsion balance but the large combs and oviducts were weighed on a triple-beam balance. Oviducts which contained fluid or coagulated material were emptied of their contents before weighing. Pituitaries were assayed for gonadotropic potency in cockerels which were kept without food and water in "chick pullmans" during the 72-hour assay period. This method was first described by Byerly and Burrows (1938). Testis weights were used as end points in this assay and the pituitaries were administered on the basis of equivalent weights of the fresh glands. Each assay chick received 4.0 mg. of anterior pituitary.

Estradiol dipropionate[3] was injected at three dosage levels, both alone and in combination with three dosage levels of testosterone propionate.[4] The estrogen also was given in combination with two dosage levels of each of five other steroids:[5] progesterone, anhydroxyprogesterone, pregnenolone, dehydroisoandrosterone, and Reichstein's Sub stance S acetate. Progesterone also was injected at three higher dosages; and the highest dosage also was given in conbination with estradiol dipropionate. The steroids were administered subcutaneously in 0.1 cc. daily of sesame oil, and an equal amount of sesame oil was injected into the control chicks.

RESULTS AND DISCUSSION

The effects produced by testosterone propionate (T.P.) and estradiol dipropionate (E.D.) are summarized in Table 1. Comb weights were not significantly increased by the 1.0 μg. dosage level of T.P. but comb sizes were doubled by the 5.0 μg. dose and were increased nearly seven times by the 25.0 μg. dose. These increases were statistically significant. Ovarian weights were decreased slightly but only when the combination of 25.0 μg. of each of the sexogens was injected was the depression in ovarian weight of statistical significance.

The oviducts were slightly stimulated by the 5.0 μg. daily of the estradiol dipropionate but the testosterone propionate had no effect when given alone at any of the three dosage levels. Oviduct weight, however, was increased when the 25.0 μg. of T.P. was given with the 5.0 μg. of E.D. and those pullets which received a combination of the 25.0 μg. of each of the sex hormones had an augmented oviduct growth. The weights exceeded those of the controls by more than fourteen times. This augmented response of the oviducts is in contrast with the behavior of the combs in the birds which received the same hormone treatment. In the latter there was a slight inhibition of T.P. action in the presence of the E.D.

[3] The estradiol dipropionate used in this study was supplied through the courtesy of Dr. Robert Gaunt, Ciba Pharmaceutical Products, Summit, New Jersey.

[4] The testosterone propionate used in this study was supplied through the courtesy of Dr. Edward Henderson, Schering Corporation, Bloomfield, New Jersey.

[5] These steroids were purchased in crystalline form from Nutritional Biochemicals Corporation, Cleveland, Ohio.

TABLE 1. EFFECTS OF TESTOSTERONE PROPIONATE AND ESTRADIOL DIPROPIONATE EACH INJECTED DAILY ALONE AND IN COMBINATION AT THREE DOSAGE LEVELS FROM THE 20TH TO 39TH DAY OF AGE ON THE WEIGHTS OF COMB, OVARY, OVIDUCT, ANTERIOR PITUITARY, AND ON ANTERIOR PITUITARY POTENCY OF 40 DAY OLD WHITE LEGHORN PULLETS

Testosterone propionate		Estradiol dipropionate			
		0	1.0 μg.	5.0 μg.	25.0 μg.
0	Comb	20[1]—242.0[2]	12—186.7*	11—210.0	10—216.0
	Ovary	100.7	107.2	99.7	96.4
	Oviduct	54.8	55.0	60.6	300.0†
	Pit. Wgt.	4.01	4.21	3.69	4.15
	Pit. Assay	8—7.64	12—6.97	—	6.69
1.0 μg.	Comb	9—251.1	13—233.1	12—237.5	10—192.0*
	Ovary	106.0	95.0	93.9	89.6
	Oviduct	64.3	59.2	62.2	235.4†
	Pit. Wgt.	3.75	3.88	3.97	4.21
	Pit. Assay	8—6.66*	11—7.52	—	7—7.27
5.0 μg.	Comb	11—479.1†	12—399.2†	13—443.1†	11—514.5†
	Ovary	10—126.9*	82.8	90.5	89.6
	Oviduct	55.9	62.7	58.7	320.8†
	Pit. Wgt.	4.21	3.86	3.57	3.92
	Pit. Assay	—	—	—	—
25.0 μg.	Comb	13—1485.4†	9—1452.2†	10—1286.0†	12—1223.3†
	Ovary	95.3	90.6	95.2	81.6*
	Oviduct	53.8	55.4	77.5*	795.8†
	Pit. Wgt.	4.04	3.84	3.96	4.13
	Pit. Assay	12—7.27	8—6.60*	—	11—6.02†

20 Day Control

No.	Comb	Ovary	Oviduct	Pit. Wgt.	Pit. Assay
20	57.7	39.4	26.7	1.60	6.60

[1] Number of animals.
[2] All weights are means given in milligrams.
* Significant at 5% level.
† Significant at 1% level.
The data were analyzed according to the "t" test and also by "analysis of variance," Snedecor (1953).

There was no consistent change in the anterior pituitary weights in the various series, and it is important to note that there was no significant depression of pituitary weight. Pituitary gonadotropic potencies also were variable but the amount of inhibition was not extensive. The pituitaries of the control pullets produced average testis weights of 7.64 mg. in the assay chicks but, although the assay values were lower in the hormone treated groups, the lowered potencies were statistically significant in only three of the eight series tested. It must be concluded, therefore, based on the ovarian and pituitary weights and on the pituitary potencies presented in Table 1, that pituitary secretion and release of gonadotropin is not significantly inhibited in pullets until daily dosages of about 25.0 μg. of the sexogens are administered.

The fact that the various dosages had little, if any, inhibitory effect is further emphasized by comparison with the data for the 20 day control pullets shown at the bottom of Table 1. Comb, ovary, oviduct, and pitui-

tary weights were markedly increased in both the control and experimental series during the interval from 20 to 40 days of age. It is significant that the pituitary gonadotropic potency of 40 day controls exceeded that of the 20 day controls and that pituitary potencies in the experimental groups were equal to or exceeded the 20 day controls except in the one group of birds which received the combination of 25.0 μg. of each of the hormones.

The notable feature of the data presented in Table 2 is a lack of augmenting effect on either comb or oviduct growth by the various steroids when they were administered with estradiol dipropionate. No combina-

TABLE 2. EFFECTS OF VARIOUS STEROIDS INJECTED DAILY ALONE AND IN COMBINATION WITH ESTRADIOL DIPROPIONATE FROM THE 20TH TO 39TH DAY OF AGE ON THE WEIGHTS OF COMB, OVARY, OVIDUCT, ANTERIOR PITUITARY, AND ON ANTERIOR PITUITARY GONADOTROPIC POTENCY OF 40 DAY OLD WHITE LEGHORN PULLETS

Estradiol	Progesterone			Anhydroxy-progesterone		Pregnenolone		Dehydroiso-androsterone		Reichstein's "S" acetete	
	0	5.0 μg.	25.0 μg.	5.0 μg.	25.0 μg.	5.0 μg.	25.0 μg.	5.0 μg.	25.0 μg.	5.0 μg.	25.0 μg.
						Comb					
0	242[1]	233	197	187	187	163*	179*	136†	230	174*	164*
25.0 μg.	216	123†	155*	106†	117*	177	198	105†	116†	127†	109†
						Ovary					
0	100.7	83.2	90.1	86.2	86.4	90.5	91.2	79.0	85.7	97.3	89.7
25.0 μg.	96.4	85.5	81.5	100.1	87.1	81.5	76.4	91.1	72.1*	81.0	75.6*
						Oviduct					
0	54.8	48.2	47.4	50.0	50.4	48.6	66.7	46.9	49.4	50.0	53.7
25.0 μg.	300.0†	179.6*	210.5	270.5	331.4	255.6	200.3*	210.5	302.8	286.3	252.9
						Pituitary					
0	4.01	3.53	3.86	3.85	3.90	4.02	4.39	3.79	3.52	3.93	4.08
25.0 μg.	4.15	4.22	3.98	3.99	3.67	3.99	3.80	3.85	3.69	3.90	3.80
						Pituitary Assay					
	6.99		7.61		6.51*		5.52†		6.52*		

Control pituitary assay = 7.64.
Water injected assay chicks (12) = 5.58.
[1] All weights are means given in milligrams.
* Significant at 5% level.
† Significant at 1% level.

tion of the five steroids with E.D. stimulated comb growth. The combination of the steroids with E.D. actually resulted in comb sizes that were below the average for the birds which received E.D. alone. The inhibition of the comb was statistically significant in eight of the ten combination groups.

Although the inhibitory effect of the steroids was less pronounced on the ovaries than on the comb, with one exception all of the birds which received the steroid hormones separately or in combination with the estradiol dipropionate had ovarian weights which were lower than those of the controls. Pregnenolone, dehydroisoandrosterone, and Reichstein's Substance S acetate when combined with the estradiol dipropionate had the greatest inhibitory effect.

It was thought that progesterone or even pregnenolone might stimulate oviduct growth, especially when administered with the estrogen. This was not the case at these dosage levels, however, as the effect of the estrogen

was inhibited in eight of the ten series. The only increment occurred when E.D. was injected with the high dose of anhydroxyprogesterone but the increase of 31.4 mg., however, is not statistically significant.

Pituitary weights were extremely variable. The lower dosage of progesterone, the higher dosage of dehydroisoandrosterone, the estradiol dipropionate plus the higher dosage of anhydroxyprogesterone, and the estradiol dipropionate plus the higher dose of dehydroisoandrosterone depressed pituitary weights but the decreases were not significant. It must be concluded, as in the case of the combinations of T.P. with E.D., that these dosages had little adverse effect on anterior pituitary growth in the pullet.

The assay of the control pituitaries and those of chicks treated with estradiol dipropionate were discussed previously. The birds which received the higher dosage of progesterone plus E.D. had pituitaries equal in potency to those of the controls. The combination of anhydroxyprogesterone with E.D. produced pituitaries which were considerably less potent than those of the controls and even slightly below those of the birds which received the estrogen alone. This also is true for the combination of the dehydroisoandrosterone and the estradiol dipropionate. The major depression of pituitary potency occurred in the series which received pregnenolone and the E.D. in combination. The testis weight average of 5.52 mg., for this group, was almost identical with that of the water injected controls which had testis weights of 5.58 mg. It should also be noted that this same series had one of the lowest ovarian weight averages and that the combs and oviducts also were among the smallest. It appears that the higher dosage of pregnenolone had considerable inhibitory effect on both the pituitary and the ovary.

The fact that the various treatments shown in Table 2 did not produce changes in combs, oviducts, or in the pituitary-gonad axis comparable with those which we had previously observed in the normal pullet at sexual maturity nor with the combinations of testosterone propionate and estradiol dipropionate shown in Table 1 suggested that a more extensive study should be made of at least one of the steroids. Progesterone was chosen for this more detailed analysis. Larger amounts of progesterone, therefore, were injected into the pullets to determine the effects of higher dosages on comb, oviduct, and ovarian weights as well as on pituitary weight and potency. An additional group of pullets also received 500 μg. of progesterone in combination with 25.0 μg. of estradiol dipropionate. Table 3 presents these data.

Attention should be directed to the fact that the controls in Table 3 differ slightly from those recorded in Tables 1 and 2. The latter were hatched in the spring whereas in the first experiment, the birds were hatched in mid-winter. Chicks are very sensitive to environmental conditions and under the laboratory situation which prevailed at the time the

study was made there were temperature and light variations which influenced the anterior pituitary and comb of the chicks. It has been imperative, therefore, to have controls for each set of experiments.

The influence of the graded dosages of progesterone when given alone on the various organ weights was not spectacular. There was some depression of comb growth but since a comparable inhibitory effect was not reported by Bolton (1953) our results may merely reflect normal variability. Ovarian weights fluctuated and actually were slightly heavier in the 500

TABLE 3. EFFECTS OF PROGESTERONE AT THREE DOSAGE LEVELS INJECTED DAILY FROM THE 20TH TO 39TH DAY OF AGE ON THE WEIGHTS OF COMB, OVARY, OVIDUCT, ANTERIOR PITUITARY, AND ON ANTERIOR PITUITARY POTENCY OF 40 DAY OLD WHITE LEGHORN PULLETS. THE HIGHEST PROGESTERONE DOSAGE (500 μG.) ALSO WAS ADMINISTERED DAILY IN COMBINATION WITH 25.0 μG. OF ESTRADIOL DIPROPIONATE

	Control	100 μg. prog.	250 μg. prog.	500 μg. prog.	25 μg. E.D. +500 μg. prog.
Number	14	15	15	15	15
Comb	314.28[1]	296.00	274.00	290.00	246.15*
Ovary	97.82	92.78	83.00	97.23	71.85†
Oviduct	53.58	55.76	53.00	62.08	2256.15†
Pit. Wgt.	4.01	3.88	4.07	3.81	3.96
Pit. Assay	14—8.60	14—7.89	14—8.22	13—9.17	13—5.69†

Mean testis weight of 15 water injected assay controls—5.37 mg.
[1] All weights in milligrams.
* Significant at 5% level.
† Significant at 1% level.

μg. than in the 250 μg. progesterone series. Oviduct weight was not significantly altered and, although anterior pituitary weights again varied, there were no significant differences following progesterone treatments. Pituitary gonadotropin, however, showed interesting variations with respect to dosage and the average potencies were higher than those of the winter chicks of Tables 1 and 2. These data again may be primarily a reflection of individual variation.

The most significant effects occurred when the 500 μg. of progesterone was administered in combination with the estradiol dipropionate. Oviduct growth was greatly augmented and the increase was far greater than an additive effect of the two hormones. Ovarian weight, on the other hand, was adversely affected and the lightest ovaries observed in any of the experiments occurred in these birds. Pituitary weight was not appreciably different from that of the controls but the potency of these pituitaries was greatly depressed; gonadotropin was almost completely absent. The decreased ovarian weight and lowered pituitary potency indicate that both secretion and release of gonadotropin by the anterior pituitary were inhibited by the simultaneous injection of the progesterone and estradiol dipropionate.

These results may now be considered in relation to the four questions posed in the introduction. It is clear that testosterone propionate alone

and in combination with estradiol dipropionate can stimulate marked comb growth in pullets. Furthermore, when T.P. was injected with 5.0 μg. and 25.0 μg. of E.D., significantly greater oviduct growth was produced than when either of the two hormones was given separately. The estrogen, however, appeared to inhibit slightly the effect of the androgen on comb growth and this finding is in harmony with the somewhat similar observations reported by Benoit (1950) and may account for the normal sex difference and comb sizes.

The five other steroids tested alone and in combination with the estrogen at the same dosage levels which were effective for T.P. actually had an inhibitory effect on combs and oviducts in most instances. The more extensive study of the action of progesterone shows also that dosages of this steroid up to the level of 500 μg. daily did not stimulate either comb or oviduct growth when given alone.

Although combinations of 5.0 μg. and 25.0 μg. daily of progesterone with the estradiol dipropionate did not augment oviduct growth, the combination of 500 μg. daily with the estrogen produced the maximum growth of the oviducts in these experiments. Hertz, Larsen, and Tullner (1947), Phillips, Common, and Maw (1952) and Bolton (1953) have reported that progesterone antagonizes the action of estradiol on the oviduct. Mason (1952), on the other hand, showed that if certain dosages of the two hormones were used, there was an augmented oviduct growth. The data reported here, therefore, re-emphasize the importance of the balance of the two hormones. Slight inhibition of estradiol action was observed at the low dosages and marked augmentation was noted at the high dosage.

It appears improbable that progesterone could have much influence on the growth and maturation of the reproductive system of the female bird. There are several observations which support this conclusion in spite of the fact that maximum oviduct growth occurred in those birds which received 500 μg. of progesterone with E.D. Comb growth and ovarian growth were markedly depressed in those birds which received progesterone and E.D. and the amount of gonadotropin in the anterior pituitaries was negligible. Fox (1955) has reported that the daily administration of from 2.0 to 16.0 mg. of progesterone partly suppressed comb and testis growth in White Leghorn cockerels and observed that progesterone delayed sexual maturity in pullets. Adams (1955) also has stated that progesterone produced molting in mature White Leghorn hens and that egg-laying stopped immediately upon injection of 13.0 mg. of hormone twice weekly. The sex behavior in the hens also was modified; for example, squatting ceased. Finally, the work of Fraps, Hooker, and Forbes indicates that although progesterone plays an important role in stimulating ovulation, it probably is present only periodically and in small amounts.

If, on the other hand, amounts of an androgen and an estrogen sufficient

to stimulate both comb and oviduct growth are present normally in pullets, the question of their inhibitory effects on the pituitary-gonad axis must be considered. The data in Table 1 are significant in this regard. Ovarian weights were very slightly affected and then only when the highest dosages of the sexogens were administered. Anterior pituitary weights were within the normal range in all experimental groups. Pituitary content of gonadotropin also was slightly affected and again the maximum inhibition occurred in the birds given the highest dosages of the two hormones in combination. It appears, therefore, that amounts of androgen and estrogen sufficient to produce marked comb and oviduct growth have surprisingly little inhibitory effects in pullets.

It is possible that the growth of the reproductive organs may be nonhormonal in chicks of this age. The work of Breneman and Mason (1951) demonstrated, however, that in cockerels the reverse is true. Anterior pituitary gonadotropin and androgen are both present in considerable amounts before 40 days of age. Pullets, of course, may develop more slowly but several features of the current data indicate that pituitary gonadotropin is present and exerting its influence. There is an increase not only in ovarian weight between 20 and 40 days of age in normal pullets but, in addition, the anterior pituitary gland increases in weight and in content of gonadotropin. Although the androgen-estrogen combinations had little adverse effect, the administration of pregnenolone, dehydroisoandrosterone, and anhydroxyprogesterone in combination with the estradiol dipropionate had much greater inhibitory effect. Finally, the progesterone-estradiol combination shown in Table 3 resulted in a marked depression of anterior pituitary gonadotropin content, a very significant lowering of ovarian weight, and a considerable drop in comb weight. Taken collectively, these results suggest that the hormones in the pituitary-gonad axis are functioning in pullets as well as in cockerels of this age. It is possible that the sexogens also may have some direct inhibitory action on the ovary but this must await the histological and histochemical study of the ovaries which is in progress.

On the basis of the data reported here, both an androgen and an estrogen appear to be present and necessary for the normal development of the female reproductive system of the bird but the effect of progesterone and of certain other steroids seems to be unimportant or even adverse.

SUMMARY

Testosterone propionate significantly increased comb weight of 40 day pullets when administered daily at dosages of 5.0 μg. and 25.0 μg. for 20 days. The stimulating effect of androgen on comb growth was inhibited approximately 25% by the simultaneous daily administration of estradiol dipropionate. Ovarian weights were only slightly inhibited by the adminis-

tration of either of the sexogens and only when 25.0 μg. daily of both hormones were administered simultaneously was the inhibition of statistical significance.

Oviduct weights were not increased by any of the three dosages of testosterone propionate alone. The injection of 25.0 μg. of estradiol dipropionate alone, however, resulted in a 600% increase in oviduct weight. When 25.0 μg. of T.P. was given in combination with 25.0 μg. of E.D., the oviduct weight was more than 1400% greater than that of the control.

The administration of progesterone, anhydroxyprogesterone, pregnenolone, dehydroisoandrosterone, and Reichstein's Substance S acetate at dosage levels of 5.0 μg. and 25.0 μg. alone and in combination with 25.0 μg. of estradiol dipropionate, did not augment oviduct growth. These steroids either alone or in combination with the estrogen, however, had greater inhibitory effects than did testosterone propionate. Comb and oviduct growth were both inhibited by the five steroids.

Pituitary weights were appreciably lower in only three of the ten steroid series. Pituitary gonadotropic content was not affected by the progesterone-estradiol dipropionate combinations but was decreased in the other series. The maximum depression of pituitary gonadotropin occurred in the pullets which received the 25.0 μg. of pregnenolone in combination with the estradiol dipropionate. The weights of the testes in the assay animals of this group were nearly identical with those of the water injected controls.

The administration of progesterone at daily dosages of 100 μg., 200 μg., and 500 μg. had very little effect on organ weights or pituitary potency. Comb weights were not increased but were slightly depressed and there was a suggestion that the amount of pituitary gonadotropin was altered by the lowest and highest dosages of progesterone. The administration of 500 μg. daily of progesterone with the estradiol dipropionate had marked effects in contrast with the slight effects produced by the low dosages of progesterone with E.D. or by the progesterone alone. The oviduct weights were 4200% greater than those of the controls but comb growth and ovarian growth were significantly lowered. Although pituitary weight was not altered, the amount of pituitary gonadotropin was negligible in these birds. It is concluded that progesterone probably plays no important role in the morphological changes which occur in the reproductive system in the female bird but that an androgen in cooperation with an estrogen contributes significantly to this development.

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THE DISTRIBUTION OF OXYTOCIN AND VASOPRESSIN (ANTIDIURETIC HORMONE) IN THE NEUROHYPOPHYSIS OF THE CAMEL[1]

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THE first serious effort to estimate the total quantity of oxytocin and vasopressin in the important hypothalamic divisions of the neurohypophysis in comparison with the posterior lobe was made some years ago by Hild and Zetler (1951–52). Although most of the subsequent observations of these authors were made in dog tissues, the distribution of the hormones in the hypothalamus and posterior lobe was also investigated in the hog and ox and in man. Similar studies, undertaken later in the dog by improved methods (van Dyke, Adamsons and Engel, 1955) have required modification of some of the conclusions reached by Hild and Zetler. These improved methods have been used for investigating the distribution of the hormones in the hypothalamus and posterior lobe of camels (*Camelus dromedarius*) from the Algerian Sahara which were used for water balance studies by two of the authors (Schmidt-Nielsen *et al.*, 1955). The unique ability of this large mammal to withstand water-deprivation suggested that the estimation of hormone-distribution in its tissues could yield interesting results which are here summarized and compared with those obtained in other mammals.

MATERIALS AND METHODS

Although the posterior lobes of 13 camels were obtained the hypothalamus could be investigated in only 9 animals. *Complete* assays of the tuber cinereum, supraoptic nuclei and paraventricular nuclei were feasible in only 5 of the hypothalami owing to the impossibility of accurate dissection or to the presence of agents interfering with bio-

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assay. Immediately after death, the pituitary body, stalk, hypothalamus and a portion of the thalamus were removed as one block of tissue and placed in 20 volumes of anhydrous acetone. This treatment was repeated 4 times before shipment, by air, of the block still immersed in acetone. On arrival, the various divisions of the hypothalamus and the posterior lobe were dissected carefully while the tissues were still damp. They were then completely dried and the neurohypophysial hormones were extracted with hot aqueous acetic acid (about 0.3%) at pH 3.0 after the tissues had been finely ground and suspended in the extracting fluid. All assays were performed in comparison with U.S.P. posterior pituitary reference standard. The blood pressure of the anesthetized rat was used for the assay of vasopressin. Antidiuretic hormone which is identical with vasopressin was determined in the trained unanesthetized dog. For the assay of oxytocin, milk-ejection pressure in the lactating anesthetized rabbit was employed but necessitated a correction for the vasopressin present as do all procedures for the assay of oxytocin. The methods all require the intravenous injection of small amounts of the hormones (1–10 milli-units, hereafter designated as mU) and have been described in detail elsewhere (van Dyke *et al.*, 1955).

RESULTS

Only in the posterior lobe was it possible to determine both antidiuretic hormone and the vasopressor effect of this hormone. The dissected hypothalamic nuclei, with which some surrounding tissue was necessarily included, all contained considerable amounts of what appeared to be the substance P of von Euler and Gaddum (1931). This substance causes a marked fall in blood pressure and vitiates the assay of low concentrations of vasopressin when this hormone is determined by its vasopressor action. Therefore, in the hypothalamic tissues, vasopressin had to be determined by its antidiuretic action which is not altered by the coincident injection of substance P. The method of determining oxytocin in the lactating rabbit likewise is not affected by the presence of substance P.

The best available information concerning the distribution of neurohypophysial hormones has been obtained in the dog and the amounts present in comparison with the camel are shown in Table 1. There is a striking difference between the two species with respect to the storage of vasopressin and oxytocin in the hypothalamus. The amount of vasopressin in all parts of the hypothalamus is about 5 to 15 times higher in the dog than in the camel. Oxytocin, on the other hand, is higher in the paraventricular nuclei of the camel than in the corresponding tissue of the dog ($P = <0.0001$). The differences between the camel and dog with regard to the amounts of oxytocin in the other parts of the hypothalamus are not statistically significant.

In the posterior lobe of the camel, the total amount of vasopressin (antidiuretic hormone) is about 8 times as great as in the dog. However, the *concentration* of vasopressin is about twice as high in acetone-desiccated canine posterior lobe (about 4500 mU per mg.) as in similarly desiccated glands of oxen or camels (about 2000 mU per mg.). The total amount of vasopressin in the posterior lobe is approximately the same (about 130,000

TABLE 1. THE DISTRIBUTION OF NEUROHYPOPHYSIAL HORMONES IN THE HYPOTHALAMUS AND POSTERIOR LOBE OF DOGS AND CAMELS

(The means and their standard errors are given as MU in terms of U.S.P. Reference Standard)

Hormone and method of assay	Paraventricular nuclei			Supraoptic nuclei			Tuber cinereum			Pars neuralis (Posterior lobe)		
	Dog	Camel		Dog	Camel		Dog	Camel		Dog	Camel	
		N*			N*			N*			N†	
Vasopressin												
Antidiuretic	460 ± 99	6	35 ± 3	1,700 ± 390	5	96 ± 23	1,900 ± 430	9	413 ± 90	16,700 ± 1,840	13	132,000 ± 10,100
Vasopressor	470 ± 104			1,600 ± 330			2,000 ± 520			15,800 ± 1,780	13	135,000 ± 11,000
Oxytocin												
Milk-ejection†	30 ± 11	6	177 ± 43	66 ± 22	5	43 ± 5	145 ± 47	9	273 ± 59	10,600 ± 1,150	13	40,000 ± 5,800

* Number of camels. There were either 10 or 11 animals in every group of dogs; the data on dogs are taken from van Dyke *et al.*, 1955.
† Corrected for milk-ejection effect of vasopressin in extract.

to 140,000 mU) in oxen and camels. There is much less oxytocin in the neural lobe of the camel than in that of the ox (about 40,000 mU compared with about 100,000 mU).

In all previous investigations of the mammalian hypothalamus or its divisions, the ratio of vasopressin to oxytocin has always been found to be high and has ranged from 3 to 24 in the dog (Hild and Zetler, 1951; Vogt,

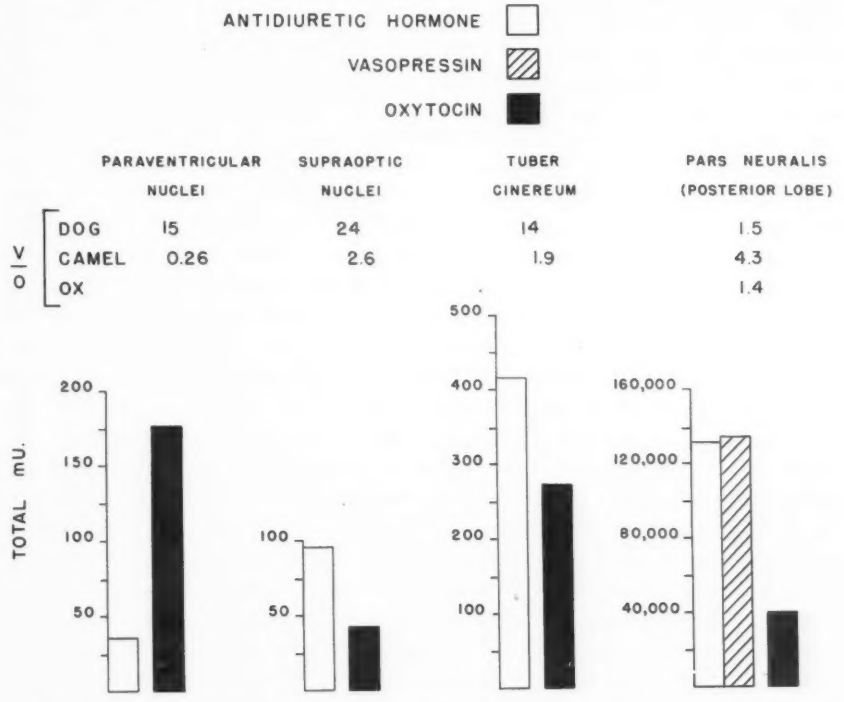

FIG. 1. The distribution of neurohypophysial hormones in the hypothalamic nuclei and posterior lobe of the Algerian camel. V/O indicates ratio of vasopressin to oxytocin in terms of U.S P. posterior piutitary reference standard. The figures given were computed as the mean from the V/O ratios of individual animals. If the ratio were computed from the mean amounts of hormones in each tissue different ratios would be obtained; for example, V/O in the paraventricular nuclei would be 0.20 instead of 0.26.

1953; van Dyke *et al.*, 1955). In the divisions of the camel hypothalamus, it is low and in one tissue, the paired paraventricular nuclei, there is about 4 times as much oxytocin as vasopressin so that the V/O ratio is about 0.25. The important difference in the distribution of the two hormones in the paraventricular nuclie in comparison with other tissues is clearly shown in Figure 1. The paraventricular nuclei of the camel contain about 6 times as much oxytocin as the corresponding nuclei in the dog. A further peculi-

arity of the storage of neurohypophysial hormones in the camel was encountered in the posterior lobe. The vasopressin to oxytocin ratio is much higher in the camel (4.3) than in the dog or ox (each about 1.5, see Fig. 1).

DISCUSSION

The camel as observed in the Algerian Sahara can withstand a much more severe dehydration that most mammals (Schmidt-Nielsen, *et al.*, 1955). Death usually occurs in mammals when about 15% of the body water has been lost although occasionally an animal will survive when the dehydration represents 20% of the body water. On the other hand, the camel can survive after the loss of more than 40% of its body water. Also, the urine flow of the camel is often very low. One camel kept on a diet of dry dates and hay and with free access to drinking water had a urine flow of 0.5 liter per day. The low volume of urine was accompanied by a high tubular reabsorption of water. It was therefore of interest to determine the amount and distribution of neurohypophysial hormones in the camel's hypothalamus and posterior lobe particularly because of the importance of antidiuretic hormone in the renal conservation of water by mammals.

Comparisons of hormone-distribution were made between the dog, ox and camel. The posterior lobes of the ox and the camel contain about the same amounts of antidiuretic hormone which is identical with vasopressin. The various divisions of the hypothalamus of camels contain about 550 mU of antidiuretic hormone in comparison with about 4000 mU in the dog. No assays of the hypothalamus of oxen were made; according to the estimate of Hild and Zetler (1952), the whole hypothalamus of the ox contains 200 mU of the hormone. In comparison with the dog, the hypothalamus of the camel is relatively rich in oxytocin. This is most strikingly demonstrated in the paraventricular nuclei in which there is 4 times as much oxytocin as vasopressin so that the V/O ratio is 0.26 compared with 15 in the dog. The V/O ratio of the paraventricular nuclei of the camel is significantly lower than the ratio in this mammal's supraoptic nuclei ($P=0.01$) or tuber cinereum ($P= <0.03$). This finding may have a bearing on Olivecrona's (1954) conclusion that, at least in the rat, the paraventricular nuclei control the production of oxytocin. The total amount of oxytocin in the camel's hypothalamus is, as in other mammals, small (about 500 mU) and about the same as the quantity of antidiuretic hormone found there (about 550 mU). In other mammals except possibly the hog (Hild and Zetler, 1952), there is relatively far more antidiuretic hormone. The relationship is reversed in the posterior lobe. There, a relatively small quantity of oxytocin is found with a V/O ratio of 4.3 compared with 1.4 or 1.5 for the ox or dog. The difference in the ratios of the camel and ox is statistically significant ($P=0.02$). The posterior lobes of other mammals have been investigated by several authors who reported V/O ratios of 2 to 3 for the

TABLE 2. CAMELS CONTRIBUTING THE TISSUES USED FOR THE BIOLOGICAL ASSAYS SUMMARIZED IN TABLE 1 AND FIGURE 1

No.	Sex	Estimated			Remarks
		Age	Weight	Hydration	
		Years	Kgm.		
1	♂	11	450	Probably normal	No water for 28 days.
2	♀	20	300	Normal	Pregnant. Near term.
3	♀	10	350	Normal	Encephalitis previously; still born fetus 1 mo. previously.
4	♂̸	16	400	Normal	
5*	♂	8	450	Normal	
6*	♂̸	11	450	Normal	No water for 45 days; very obese.
7*	♂̸	9	500	Normal	No water for 60 days.
8*	♂̸	8	400	Normal	
9	♀	16	350	?	Poor teeth; emaciated.
10*	♀	10	380	Normal	
11	♂̸	16	450	Dehydrated	No water for 6 days; estimated deficit: 60 l. of water.
12	♀	12	350	Normal	Pregnancy of 4 mos.
13	♂̸	8	500	Normal	

♂̸ = castrated male.
* Complete analyses of hypothalamus.
Camels 1, 6 and 7 obtained water from fodder; in neither these camels nor in camel 11 were there conspicuous changes in the amount of hormones in the posterior lobe.

guinea pig, rabbit, wallaby and finback whale and 12.5 for the sperm whale. Very high ratios have been found in fetal posterior lobes (Dicker and Tyler, 1953).

The amount or distribution of the hormones in the hypothalamus or posterior lobe of individual camels could not be correlated with sex, pregnancy, age or dehydration (1 camel) and the pertinent information is summarized in Table 2. Only stored hormones could be determined and their amounts do not furnish reliable clues concerning the rate of either their formation or their liberation except in the paraventricular nuclei which are remarkably rich in oxytocin. No evidence favoring a special physiological importance for antidiuretic hormone in the camel was obtained.

SUMMARY

The total amount of antidiuretic hormone (vasopressin) in the neurohypophysis of the camel is similar to that found in oxen. Only *stored* antidiuretic hormone could be investigated and no evidence suggesting a special role of the hormone in water conservation in the camel can be offered.

Peculiarities in the distribution of oxytocin were noteworthy. The relative concentration of the hormone was remarkably high in the paraventricular nuclei with a vasopressin/oxytocin ratio of 0.26. On the other hand, an unusually small amount was found in the posterior lobe (40,000 mU) with a V/O ratio of 4.3 in comparison with 1.4 for the same structure in the ox.

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NOTES AND COMMENTS

THE IODINE METABOLISM OF THE THYROID OF ANDROGEN-TREATED RATS[1]

The administration of testosterone propionate to rats produces an initial increase in the rate of body growth and then a marked decrease (Kochakian, Robertson and Bartlett, 1950). Since a number of reports (cf. Kochakian and Dolphin, 1955) suggest a thyroid-androgen interrelationship, it seemed worthwhile to determine whether the androgen would influence the iodine metabolism of the thyroid of rats.

PROCEDURE

Rats of the Sprague-Dawley strain were maintained in individual metal cages in an air-conditioned room at 25.5–26.6° C. and with constant artificial light 12 hours per day. They were fed *ad libitum* a prepared diet (cf. Kochakian and Dolphin, 1955) with a calculated iodine content of 0.14 mg./100 gm.

The rats were castrated at 106 days of age (340–435 gm.) and 36 days later were divided into 2 groups of 10 rats each. One group was injected subcutaneously with 1.0 mg. testosterone propionate[2] per day and the other 0.1 ml. sesame oil per day for 38 days. Two groups of normal rats of the same age received similar injections. All of the rats were injected intraperitoneally with 5 μc. I^{131} in 1 ml. saline at 7 days before and at 10, 20 and 39 days after the beginning of the androgen injections. The radiation of the thyroid gland of each animal was measured 48 hours after the I^{131} injection and daily thereafter with a collimated scintillation gamma detector. The rat was held so that the crystal of the detector was approximately 8 cm. from the thyroid area. Animals which had initial counting rates of less than 1,000 counts/minute were discarded. Net counting rates were determined by subtracting background measurements and radiation from any residual I^{131} activity in the thyroid gland versus time. The relative thyroid uptake of I^{131} was determined by comparing the average daily net counting rates of each group at 48 hours with that of the normal rats. The fractional disappearance of thyroidal I^{131} was computed by taking the average of the daily disappearance rate corrected for physical decay.

RESULTS

No difference was observed in I^{131} uptake by or release from the thyroid (Table 1) as a result of castration or as a result of the injection of the androgen in the normal and castrated rats.

The apparent difference in disappearance of thyroidal I^{131} between the normal and castrated rats in the control period was not supported by the control values of the castrated plus testosterone propionate group.

DISCUSSION

The failure of castration and androgen treatment to influence the iodine metabolism of the thyroid gland suggests that thyroxine is not involved in the various aspects of the growth stimulating and inhibiting actions of testosterone propionate in the rat. The administration of testosterone propionate to mice also has been noted to be ineffective on thyroidal iodine metabolism (Brands and Montag, 1953). On the other hand, there is histological evidence that testosterone propionate affects the thyroid gland of rats (Grunt and Leathem, 1949), and beef (Burris, Bogart and Krueger, 1953). Indeed, the

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[1] This investigation was aided by an Institutional Grant from the American Cancer Society.

[2] The testosterone propionate was supplied as Perandren, 10 mg./ml., by Ciba Pharmaceutical Products, Inc.

TABLE 1. THE IODINE METABOLISM OF ANDROGEN-TREATED RATS[1]

Rat group	Control period	Injection periods		Post-injection period
		1	2	
	Relative thyroid uptake of I^{131}			
Normal	1.00	1.00	1.00	1.00
Normal+T.P.	.80	1.04	1.17	1.09
Castrate	1.08	0.87	0.94	0.88
Castrate+T.P.	.95	1.07	1.20	1.16
	Fractional disappearance of thyroidal I^{131} per day			
Normal	.208	.200	.210	.205
Normal+T.P.	.200	.212	.209	.198
Castrate	.161	.184	.173	.196
Castrate+T.P.	.187	.194	.195	.182

[1] Ten rats per group. Each rat was injected intraperitoneally with 5 μc. I^{131} in 1 ml. saline at 7 days before (Control) and at 10, 20 and 39 days (Injection periods 1 and 2, and Post-injection period respectively), after the beginning of testosterone propionate injections of 1 mg./day for 38 days.

growth stimulating effect of androgen in beef has been suggested to be mediated through the thyroid.

SUMMARY

The I^{131} uptake by or release from the thyroid was not affected by castration or the administration of 1 mg./day of testosterone propionate for 38 days to normal or castrated rats.

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EFFECTS OF HYPOPHYSECTOMY ON THE COENZYME A CONTENT OF RAT LIVER

Recently, Ringler and Leonard (1954) reported that hypophysectomy reduced the coenzyme A (CoA) content of the liver. They hypophysectomized adult rats and determined the liver CoA content 10 days later. Cortisone acetate, growth hormone, thyroxine, and pantothenate injections were found to increase the liver CoA level.

In the current study the CoA content of the liver was investigated 166 days after

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hypophysectomy. The pituitary was removed from male Sprague-Dawley rats when they were 23 days old. The operation and subsequent care has been described by Scow (1953), to whom we are indebted for the animals. Operated rats and unoperated age-controls were maintained on a modification of McCollum's lactating diet for 166 days, at which time the animals were 189 days old and weighed, on the average, 100 and 402 gm., respectively. At sacrifice, examination of the region of the *sella turcica* confirmed the completeness of the hypophysectomies. A group of male rats of the same strain that had been fed a commercial stock diet from weaning to 40 days of age and weighed about 93 gm. was used as the weight-control.

The results (see Table 1) clearly show that the liver CoA concentration of hypophysectomized rats did not differ from that of unoperated controls of the same weight, and was greater than values found for controls of the same age ($p < 0.01$). A metabolic manifestation of hypophysectomy was, however, observed in the lowered Q_{O_2} of liver slices from operated rats (18 per cent less than age-control values). Thus, neither the pituitary hormones nor the metabolic rate seem to be primary factors in determining the concentration of liver CoA. The importance of the general nutritional status of the animal in the maintenance of normal concentrations of the coenzyme has been reemphasized recently (Chernick, Moe, and Schwarz, 1955).

SUMMARY

The concentration of liver CoA was measured 166 days after hypophysectomy of male Sprague-Dawley rats. The CoA concentration was maintained at the juvenile level and was greater than that of age-controls.

TABLE I. EFFECT OF HYPOPHYSECTOMY ON CoA CONTENT OF LIVER

Treatment	Number of rats	Age (days)	Body weight (gm.)	CoA content of liver[1] (μM./gm.)
H at 23 days of age[2]	10	189	100	$0.42 \pm .08$[3]
Unoperated age-controls	8	189	402	$0.33 \pm .02$
Unoperated weight-controls	12	40	93	$0.45 \pm .09$

[1] Determined by the method of Stadtman (1953). 317 Lipmann units=1 μM. CoA.
[2] Hypophysectomy performed by Dr. R. O. Scow.
[3] Mean and standard deviation.

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THE EFFECT OF 17 ALPHA-HYDROXY-11-DESOXYCORTICOSTERONE ON ESTROGEN-STIMULATED CHICK OVIDUCT GROWTH

In an earlier study, Hertz *et al.* (1947) reported on the marked inhibition of estrogen-induced oviduct growth in the chick when progesterone was administered simultaneously with either estradiol or stilbestrol.

Later, allopregnane-21-ol-3, 20-dione acetate was found to be more active than progesterone in the chick oviduct assay but completely inactive using several other common methods for the assessment of progestational activity (Tullner *et al.*, 1954).

The present report compares the activity of 17 alpha-hydroxy-11-desoxycorticosterone 21-acetate (Reichstein's Substance S acetate) with that of progesterone.

MATERIALS AND METHODS

Female New Hampshire Red Chicks were maintained under controlled lighting (12 hours light, 12 hours darkness) on a commercial starting mash and water *ad libitum*. At 2 weeks of age the chicks were started on daily Subcutaneous injections of 0.25 mg. stilbestrol in 0.1 cc. corn oil. Progesterone or Substance S acetate were given concurrently with stilbestrol as suspensions in corn oil. The treatment period was 8 days. Twenty-four hours after the last injection, the chicks were autopsied. Body and oviduct weights were recorded.

RESULTS AND DISCUSSION

The adrenal cortical compound 17 alpha-hydroxy-11-desoxycorticosterone has a pronounced inhibitory action on estrogen-induced growth of the chick oviduct (Table 1).

In other experiments in which we have compared lower levels of Substance S and progesterone, it is apparent that progesterone is the more potent steroid. Nevertheless, it can be seen in Table 1 that daily administration of 5 mg. of Substance S results in a marked oviduct weight decrease without a corresponding effect on body weight.

TABLE 1. EFFECT OF SUBSTANCE S* AND PROGESTERONE ON CHICK OVIDUCT RESPONSE TO ESTROGEN

Steroid	Daily dose (mg.)	No. of animals	Terminal† body wt. (gm.)	Oviduct† wt. (mg.)
Substance S	1	13	200 ± 23	825 ± 111
Progesterone	1	11	209 ± 21	578 ± 86
Substance S	5	9	216 ± 20	590 ± 115
Progesterone	5	5	189 ± 21	468 ± 35
O (stilbestrol only)	0	10	208 ± 17	1122 ± 199

* Dosage stated as acetate.

† Mean and standard deviation. Mean oviduct weight for untreated chicks of the same age and body weight is 22 mg.

The inhibition of oviduct growth by Substance S is in marked contrast to its progestational action. Using the Clauberg rabbit assay in which a 0.5 mg. total dose of progesterone will produce full progestational proliferation of the endometrium, 400 times that amount of Substance S was required for a comparable response. Desoxycorticosterone acetate shows a marked inhibition of estrogen-induced chick oviduct growth (Tullner *et al.*, 1954) in addition to a progestational action about 1/10 that of progesterone (Miescher *et al.*, 1938) whereas substance S has no effect in the Clauberg test at a dose of 10 mg.

Huggins and Jensen (1955) report a substantial inhibition of estrone-induced uterine growth in the hypophysectomized rat using 0.25 mg. daily doses of progesterone and

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DOC. The chick oviduct experiments have indicated similar effects. Experiments designed to determine minimal amounts of steroids required to inhibit development in the chick embryo show the amount of steroid required per egg to be the same both for progesterone and Substance S, although only 1/20 this amount of DOCA is required for inhibition (Stock, 1952). Our data are in agreement with these findings insofar as growth-inhibiting properties are concerned although one cannot make comparisons on a quantitative basis.

However, there are differences in the actions of these compounds on other types of tissue growth. In experiments described by Lipschutz *et al.* (1952) on the comparative antifibromatogenic action of cortical steroids, it was found that quantities of Substance S about 7 times those of DOC had no antifibromatogenic action. Both progesterone and DOC are potent antifibromatogenic agents.

This marked oviduct growth inhibition by an adrenal cortical compound in contrast with its minimal progestational activity again demonstrates that progestational potency is not necessarily concomitant with the ability to inhibit estrogen-induced oviduct growth.

SUMMARY

17 alpha-hydroxy-11-desoxycorticosterone acetate (Reichstein's Substance S acetate) has a marked inhibitory effect on estrogen-induced chick oviduct growth. This inhibition is not associated with progestational activity.

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OVERTURE TO 1956*

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THERE is a play by the brothers Quintero in which an old man seeks to bring his warring progency together for an occasion that seems to him very important. For on this particular day he was to be 100 years old. We can ourselves tonight pretend to be a hundred years old if such fancies give us pleasure. We can at least celebrate the centenary of our name year, even if the birth year of our subject is more ancient and obscure. In 1855 Claude Bernard in a lecture at the College of France used the phrase "internal secretion" for the first time. We ourselves were once the Association for the Study of Internal Secretions. We now have a shorter name that means much the same thing. We lack of course the power or the influence of the Spanish patriarch to assemble our children. Those of us who are here, however, can try to pass a pleasant evening thinking of a hundred years and more. To do so I shall draw most heavily on the biographies by Professor Olmsted of the University of California, and paraphrase a familiar story told their students by all teachers of Endocrinology.

In 1855 the year of our pretended name year Claude Bernard assumed the Chair of Medicine in the College of France following Francois Magendie. That same year thinking of the secretions of sugar by the liver which he had demonstrated he used the term "internal secretion" as we have noted earlier. The idea was not new. Bordeau's theories of 1776 come closer to the concept of secretions as hormones. Berthold's experiment of 1849 in which a transplanted testis sustained the combs of capons had been interpreted as meaning that an influence was exerted by this gland through the blood stream on other tissues. Mere inspection of the structure of several glands together with a little thought could hardly have yielded anything other than the view of operation through the blood stream, by ex-

* Presidential Address, Endocrine Society, delivered at Atlantic City, N. J. on June 3, 1955. An explanatory note is desirable. The reader should recall that this talk was written to be heard rather than to be read. Both the audience and the speaker had had a pleasant evening with good food and drink. Due honors had been awarded. Many of the countless spring orations deal with what should be done or with what should not be done. Being unreliable on such matters the speaker felt it wiser to try to state a particular man's place in the life of his subject and touch on what society and our society in particular had done with him. So to Brown-Séquard. No scholarship was involved. Professor J. M. D. Olmsted has kindly allowed this somewhat reckless use of his books, which are hereby recommended to those few who do not know them. The peroration however naïve and school-boyish it may sound and however much the speaker depended upon the indulgence of a pleasantly befogged audience was seriously meant. It is upon the use of all knowledge as befits a physician that the survival of man depends. At this time small voices should be added to the great ones speaking thusly so that all may hear.

traction from the blood or by secretion into it. But the mind of man moves slowly and the actual manipulation of material is required before one grasps what has been before him all along. The sugar of Claude Bernard was measurable, the contrast between bile moving outward from the liver through the ductules of the biliary tree and sugar moving inward into the blood stream itself was clearly put. Since we pick our way through chaos by giving names to things the term "internal secretions" was most welcome. Recognition of our subtler hormones was to come somewhat later.

Among the candidates for Magendie's chair was one Brown-Séquard. Against Bernard he had no chance. Yet he had his own proper claims. Indeed the first Montyon Prize in experimental medicine given by the committee under the new chairmanship of Claude Bernard went to Brown-Séquard in 1856. This for work on the transmission of sensations in the spinal cord, work still recalled for us today by the eponymic, Brown-Séquard syndrome. Thus the neurological research expressed first in the medical thesis of 1846 and matured later came to due recognition. 1855–56 marked as well the first appearance of our endocrinologist, Brown-Séquard, and permits us once again to celebrate a centenary.

Addison's monograph of 1855 is still widely quoted, "The Treatise on Constitutional and Local Effects of Disease of the Suprarenal Capsules" summarized the work of many years. These phrases are well known to you: "The patient in most of the cases I have seen has been observed gradually to fall off in general health, he becomes languid and weak, indisposed to either bodily or mental exertion; the appetite is impaired or entirely lost, . . . ; the pulse small and feeble, or perhaps somewhat large, but excessively soft and compressible; . . . slight pain or uneasiness is from time to time referred to the region of the stomach and there is occasional actual vomiting, which in one instance was both urgent and distressing; and it is by no means uncommon for the patient to manifest indications of disturbed cerebral circulation; . . . we discover a most remarkable and so far as we know, characteristic discoloration taking place in the skin; . . . this discoloration pervades the whole surface of the body. . . . It may be said to present a dingy or smoky appearance or various tints of amber or chestnut brown." So it goes with many important phrases of this classic here omitted. The disorder so described was attributed to the destructive disease of the adrenal glands found at autopsy.

Soon after Addison's account appeared Brown-Séquard attempted the experimental counterpart. He reported to the Academy of Sciences in Paris on August 25, 1856 that removal of both adrenals caused death within 12 hours, at least in cats, dogs, rabbits and guinea pigs. Removal of one gland alone did not suffice. Death occurred more rapidly than after removal of the kidneys. And the neurologist speaks out, "These organs have numerous relations with cerebro-spinal centers." Trousseau who had been Brown-Séquard's teacher reported this new work to the Academy of

Medicine, described a patient of his own with the bronze disease of Addison, a patient who at autopsy showed cretaceous material in the adrenal glands. Surprisingly enough these glands were essential to life, they were not embryonic vestigia as had been supposed. They could be conceived either as removing some toxic principle from the blood or as supplying something vital to it.

Perhaps never before or since has a clinical discovery brought its experimental confirmation quite so quickly as in this sequence of Addison and Brown-Séquard a hundred years ago. Yet all were not content. An auditor of Trousseau's account at the Academy of Medicine, one Bouillard, spoke with appreciation of the work of Addison and Trousseau but described Brown-Séquard's contribution as a philosophical prologue containing "amusing physiology." Brown-Séquard's own angry rejoinder proclaimed the value of the impoverished science of physiology. Later he was to state more formally his appreciation of the interplay between clinical and experimental medicine. Then as now everyone did not like this particular circumstance of our existence, or perhaps did not like the people who did the other thing.

Who was Brown-Séquard? He was many things and of great interest.

Some little time before 1817 when this place was a spit of sand Charles Edward Brown of Irish origin but a native of Philadelphia took ship as a captain in the American Merchant Marine. Disembarking on the tropical island of Mauritius as the Dutch had called it, or Isle de France, 550 miles east of Madagascar, on the trade route to India, he met and married Charlotte Séquard of strictly French descent. Three years later there was a famine on the island. Brown went to India to procure rice. He did not return. His son Charles Édouard Brown was born posthumously in 1817, a British citizen, since the French had yielded Isle de France to the English. Charlotte Brown made and sold embroideries and with the help of relatives and friends raised the boy. The boy was very bright and inspired by a local artistic set, wrote poems and plays. When he was 17, a collection by the town sent the boy and mother to Paris to complete his education. This was in 1838. So four years after Claude Bernard, Charles Édouard Brown went to Paris, like Claude Bernard to seek his fortune there, like Claude Bernard bringing poems and plays, and once again like Claude Bernard was told to earn his living. He took up medicine, learned physiology in the laboratory of Martin-Magron and clinical medicine in part from Trousseau. The way was very hard. He went to bed at 8:00 P.M. and arose at 2:00 to 3:00 A.M. to work in the quiet hours. He scorned all theater and opera. He asked much of himself and came thus to ask the question, which he was to ask many times. Why, in effect, cannot this go on unresting? What after all is the nature of the energies of men?

To mark himself apart from all the other Browns, Charles Édouard Brown became Brown-Séquard. And when his mother died and this tie was loosened, he became a wanderer for this and other reasons.

Between 1851 and 1854 he delivered lectures and practiced medicine in New York, Boston and Paris, and while in Boston married Ellen Fletcher the niece of the first wife of Daniel Webster. In 1854 the black arm of Asiatic cholera extended out from India as it had so many times before and embraced the island of Mauritius. Brown-Séquard was there. He engaged in the struggle with the epidemic, prescribing opium as Magendie taught him and testing the efficacy of this drug upon himself after swallowing the vomitus of cholera victims. The opium made him ill.

In 1853 the faculty of the medical school of Hampden-Sidney College of Richmond, Virginia seceded from its own board of trustees, an example all too rarely followed, and became the Medical College of Virginia. Brown-Séquard was there soon after. He demonstrated experimental procedures in the cellar of the Egyptian building on animals collected by his students through the town, lectured unintelligibly in his bare English, expressed unsolicited opinions on the slavery question, went to bed at 8:00 P.M. no matter whom the guests might be and once in the interests of science, varnished his skin completely with some unnamed material. This led to unconsciousness and collapse. He was rescued by a student. The means are now uncertain. Both alcohol and sandpaper have been suggested. In any event Brown-Séquard's resignation from the faculty was accepted and he returned to Paris for our events of 1855–56.

He did not stay in Paris very long. We find him lecturing in England and America, a colleague of Oliver Wendell Holmes and Louis Agassiz on the Harvard faculty, chiding students on the blindness of America to physiological research. We find him in fashionable practice in Wimple Street, London and at some time or other, seeing the numerous horse-drawn carriages of patients before his door, disappearing. Yet his prescription of bromide for epilepsy was a useful innovation. We find him a consultant to Thomas Addison on a neurological case in London, we find him introducing Hughlings Jackson to neurology in a London hospital. We find him in Boston once again tending Louis Agassiz in that great geologist's last illness. His own research went on but with eccentric trends. He perplexed Darwin and the natural selectionists by a curious account of the inheritance of epilepsy induced in guinea pigs by injuries to nerves, inheritance therefore of an acquired character. He opposed the rising doctrine of cerebral localization in bitter battles with his old friend Charcot. His facilities were small and when Henry Bowditch came to work with him in Paris there was no space. So Bowditch moved on elsewhere before returning to found the first American physiological laboratory in a Boston attic in 1871. In 1878 on the death of Claude Bernard we find him, a French citizen at last assuming the great one's chair as Professor of Medicine at the College of France. As always it was after Claude Bernard.

Brown-Séquard was then 61. His demands upon his strength still remained and while still preoccupied in the laboratory with the nervous system, he came to dwell more and more on the phenomena known to

him as "nervous force," the source of energies of men. Since at least the age of 44 he had been jotting down notations of his own muscle strength on a hand dynanometer. By the age of 72 his fatigue was such that he decided to deal with it directly. His propensity to work upon himself you will recall. The doctrine of internal secretions crystallized in his mind. The idea of a gland adding something to the blood stream grew more important than the alternative of taking something from it. This was 43 years after his experimental adrenalectomies brought his first consideration of a gland's influence on the blood stream and 44 years after Claude Bernard had spoken of internal secretions in this same faculty, but in the sense of a directly measurable substance. The concept of assessment of an agent's properties by biological response alone and the concept of searching for this agent in the gland itself were emerging clearly now.

D'Arsonval, the assistant of Brown-Séquard, as he had been of Claude Bernard, made watery extracts of the testes of dogs and guinea pigs and Brown-Séquard injected these into himself. The results were stated as follows in 1889 with many elisions of my own:

"I was 72 years old the 8th of April. My general vigor which had been considerable has diminished notably and gradually during the last ten or twelve years, . . . After three or four hours, and sometimes after only two hours of experimental work at the laboratory, although I sat down, I was left exhausted. On returning home by carriage about six o'clock in the evening, after some hours passed thus in the laboratory, I was for many years so tired that I had to go to bed almost as soon as I had taken a hasty meal. . . . Today and since the second day and above all the third day after the first injection, all that has changed and I have regained at least all the force which I possessed a number of years ago. Experimental work at the laboratory tires me little now. . . . I can also now without difficulty and almost without thinking about it, go up and downstairs almost running, a thing which I always did before the age of sixty. By using the dynanometer I have established that there has been an incontestable increase in the force of my limbs."

With this report the tumult then began. D'Arsonval worked incessantly to make his extracts which went everywhere in Europe. I can best quote the biographer, Professor Olmsted. "From outposts like Bucharest and St. Petersburg enthusiastic reports began to come in; the extract hastened transmission of sensory impulses, and overwhelming number of locomotor ataxia victims were helped; there was immunization against tuberculosis, augmentation in the vigor of the fetus of a syphilitic mother and twenty one cases of cancer had been ameliorated. Before the end of the year more than twelve thousand physicians were administering the extract to their patients."

Brown-Séquard interpreted the improvement often noted in no specific sense but to the increase in "nervous force" with which he had been con-

cerned. In this he was quite correct if in error about the means. The answer lay more nearly in that sphere that William James was to deal with so eloquently in his classic speculation on the energies of men—to hope and to faith, arising here from suggestion, brought to focus by a sense of peril and translated thence into a sense of well-being and into power for action, however brief. Whether humoral factors enter into this cortical response to danger, we can leave as an important question unanswered still. Something probably could be made of stimulation by the noxious extracts given, acting through the adrenal cortex or otherwise. Something more perhaps could be made of misinterpretation, the physicians rather than the patients being those suggestible. In any event Brown-Séquard's experiment was most successful as a public drama. There was much derision and much contempt expressed by sober men. Brown-Séquard himself, however, was never shaken; from this conviction was to come a new statement of the doctrine of internal secretions, or a restatement, if you will, of Bordeau's older theories of a hundred years before.

"I say now merely that all glands with an external secretion, have at the same time, like the testicles, an internal secretion. The kidneys, the salivary glands, the pancreas are not merely organs of elimination. They are like the thyroid, the spleen, etc., organs giving to the blood important principles, either in a direct manner, or by resorption after their external secretion" . . . and again "We admit that each tissue and more generally each cell of the organism secretes on its own account certain products or special ferments, which through this medium, influence all other cells of the body, a definite solidarity being thus established among all the cells through a mechanism other than the nervous system . . . all the tissues (glands or other organs) have thus a special internal secretion" . . . and again "We believe in consequence that all tissues can and should be employed in special cases as a mode of treatment; that in a word there must be created a new system of therapeutics, the medicaments of which will be products made from the different tissues of the organism. The field open to research along this path is immense."

This was quite discerning and still quite wild. One can hardly picture Claude Bernard yielding to such conjecture. And yet D'Arsonval at the instance of Brown-Séquard was attempting extracts of the pancreas for the cure of diabetes and had before him recommendations to extract suprarenal and thyroid glands as well as salivary glands, brain and spinal cord. In 1891–1892 the relief of myxedema by administered thyroid started the point to point replacement of our better science. In part through Brown-Séquard in 1889 the thing was in the air. Refinement was to come.

Now as for Brown-Séquard himself. From time to time he arose in the Academy of Sciences to say that such and such report confirmed what he had known all along and he was often right. A visitor from always grateful Mauritius found him in 1891, "a gentle, modest, white haired old man,

rather below the middle height, of manners quiet and unassuming and a smile full of kindness." He died in 1894, observing himself to the end and taking testicular extract as long as possible. And the assistant D'Arsonval, who had received his lectureship in biophysics at the hands of Brown-Séquard grew into his own great renown.

This has been a narrow treatment of the events in our history that have a degree of center in our name year of 1855. One could do as much or more from many another focus than this particular one on Brown-Séquard. One can think of the following hundred years in many ways. That from the standpoint of Brown-Séquard will do for tonight. The neglected subject of physiology he espoused is now well appointed. The movement of the mind back and forth from disease in the natural life of man to the experiment is now the usual thing. The life supporting extracts of the supra-renal glands proposed by Brown-Séquard came in 1930 but only now has the isolation of aldosterone shown that the mysteries of the water soluble amorphous fraction are being penetrated. The subject of the adrenal steroids has had a mightly growth. The widely ramifying influences of these materials in generalized disease have astounded nearly everyone. Perhaps they would not have so surprised Brown-Séquard. The varied and uncertain influences of these materials on the nervous force on man would have touched him very closely. And some of the fantastic elements in this more recent story he would have understood. Addisonians can now be supported, but this is new; only now does it appear that the characteristic pigmentation so well described by Addison a hundred years ago may be on its way to proper explanation. The testis extract sought by Brown-Séquard did not come until 1927 and has had a more measured development in application than his own, but not without some reminiscent flourishes. Watery extracts like those he used, however, may well be worth some further careful work. Effective thyroid extract that he planned to look for came while he lived, but elaboration of our understanding of this secretion, of how it is made, how delivered and how it works is still going on. The infringement of the parathyroids on the events after thyroidectomy is now understood but the nature of this parathyroid secretion itself remains obscure. The insulin he sought came in 1921. Small voices say that the pineal gland is not merely a vestigial structure as the adrenal was supposed to be a hundred years ago. More and more tissues are found to exert influences of hormonal or near hormonal kind and the brain is among them. The integrative play of the endocrines that Brown-Séquard discerned is now appreciated and a system is being mapped in all its complex order, piece by piece, that rivals in its own diffuse architecture, with its own perfected hierarchies, the excellence of that more compact one, the nervous system, that he loved to study. And the mode of interrelation between these two systems is a present great concern. But the answers to Brown-Séquard's great question: What is the nature of the nervous force of man, of

inspiration and of ardor and of long endurance? These answers he gave poorly. We give them poorly still.

So for any science so concerned with stress as ours is now, Brown-Séquard could very well be a patron saint. With him it was always more and more. If the time ever comes when the mechanisms of recovery and repose take our time we would need to seek some less impatient figure to invoke.

Now the adventure of Brown-Séquard in ill-placed glandular treatment is held to have provoked a mad use of many kinds of useless agents and to have given Endocrinology a very bad name. This may well be true and this charge brings us home. For a founder of our own society in 1917 was a purveyor of such inane glandular material used in hope and with reported benefit. But there were others with him who cared, perhaps not more for the truth as an end, but more for truer ways toward the truth. Small though their science was beside our present scope they prevailed and you are here. Refinement then has come and through your hands and those of many others a living branch of science has evolved for a hundred years and more. As we to Brown-Séquard so to us in turn. For each small truth that is in anyone of us will be found by others, be conserved and be enlarged and each mad error will be curtailed and if nothing good can be made of it pruned away. So works the good Society and ours is a fragment of it. So until the day when knowing what he needs to know and using all knowledge as befits a physician man himself shall stand erect at last and unafraid and build his true dominion in the sun.

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